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BEHAVIORAL, COGNITIVE, AND GENETIC FACTORS UNDERLYING SOCIO-COMMUNICATIVE DEVELOPMENT IN BONOBOS

by

SARA A. SKIBA

Under the Direction of Jessica Turner, PhD and David Washburn, PhD

ABSTRACT

Although it is clear that behavioral, cognitive, and genetic factors all contribute to socio-communicative development in humans, it remains a significant challenge to disentangle the contribution of each to the emergence of socio-communicative abilities. Recent research has demonstrated that single nucleotide polymorphisms (SNPs) are linked to social behavior and cognition in humans and nonhuman mammals. Bonobos, one of the species most closely related to humans, exhibit complex socio-communicative behavior and cognition, and exhibit similar connections between genetic factors and individual-level social behavior to those observed in humans. This study is the first comprehensive assessment of the behavioral, cognitive, and genetic underpinnings of socio-communicative development in a nonhuman great ape species.

Specifically, I aimed to assess the relation among social behavior, communication, repetitive/abnormal behaviors, and social cognition at the individual level. In addition, I aimed to determine whether or not SNPs associated with autism spectrum disorder (ASD) in humans are present in bonobos, and if they are predictive of individual-level socio-communicative behavior and social cognition abilities. To this end, I collected behavioral data from 26 captive bonobos, as well as cognitive task performance data from 7 of these individuals. Analyses revealed a significant negative correlation between sociality and repetitive/abnormal behaviors in female bonobos. Additionally, results indicated that communicative production was negatively correlated with completion time on a receptive joint attention task. Furthermore, this study provides the first evidence of a potential SNP in the bonobo oxytocin receptor (*OXTR*). Collectively, these findings suggest that bonobos may be an ideal model for the complex behavioral and cognitive phenotype associated with neurodevelopmental disorders, such as ASD. Indeed, this study fills a critical gap in our understanding of the various behavioral, cognitive, and genetic factors underlying socio-communicative development in humans, and our closest living relatives.

INDEX WORDS: Social behavior, Communication, Social cognition, Genetics, Development

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by

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Georgia State University

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1 INTRODUCTION

This review focuses on the behavioral, cognitive, and genetic factors underlying socio-communicative development in humans and our closest living relatives – chimpanzees and bonobos. The purpose of this review is to highlight the critical connection between social behavior, communication, and cognitive functioning, as well as to identify potential biological pathways underlying socio-communicative development in humans, bonobos, and chimpanzees. The first section, the co-evolution of social behavior and communication, focuses on the emerging evidence of a positive relationship between social behavior and communication within the primate lineage. The second section includes an approximate timeline of typical and atypical social cognition development in humans. Specifically, this section focuses on the foundational cognitive skills that incorporate social and communicative components and that are necessary for typical socio-communicative development in humans, bonobos, and chimpanzees. For the final section, I utilize a single gene to illustrate the pathways in which biological factors may influence great ape socio-communicative development.

1.1 The Co-evolution of Social Behavior and Communication

Human social behavior and language represent the most elaborate socio-communicative system in the animal kingdom. However, the selection pressures leading to its emergence are still unknown. A theory with growing support, the social complexity hypothesis for communicative complexity (SCHCC), proposes that complex communicative strategies evolved as a consequence of living in large, dynamic social environments (Bouchet, Blois-Heulin, & Lemasson, 2013; Freeberg, Dunbar, & Ord, 2012; Krams, Krama, Freeberg, Kullberg, & Lucas, 2012). Similarly, the social brain hypothesis (SBH) proposes that the social and cognitive demands of living in groups of 100 or more individuals - which likely occurred within the

hominin lineage - necessitated unprecedented complex communicative strategies (Dunbar, 1998, 2009). Collectively, these theories hypothesize that elaborate communicative behaviors, and the cognitive capacities underlying them, evolved as a tool to facilitate social bonding and navigate complex social environments.

Although there are a number of studies documenting the social and communicative behavior of monkeys, apes, and other mammals (Call & Tomasello, 2007; Cheney, Seyfarth, & Smuts, 1986; Goodall, 1986; Seyfarth, Cheney, & Marler, 1980a, 1980b), this review will focus only on literature that investigates the *connection* between social behavior and communication. Indeed, there is evidence of a positive relationship between social behavior and communication in several bird, rodent, and marine mammal species (May-Collado, Agnarsson, & Wartzok, 2007; Blumstein & Armitage, 1997; Freeberg, 2006). However, the most relevant support comes from studies with primates (Fan et al., 2018; McComb & Semple, 2005; Skiba, 2017).

For example, Gros-Louis (2002) investigated the social and communicative behavior of white-faced capuchin monkeys. Specifically, Gros-Louis (2002) found that infants that produced trill vocalizations upon approaching a conspecific were more likely to engage in affiliative behaviors (e.g., grooming, playing, climbing on) subsequent to the approach, as compared to infants that did not produce trill vocalizations. Additionally, infants were less likely to receive aggression from conspecifics if they produced a trill vocalization upon their approach, than if they did not produce one (Gros-Louis, 2002). These findings are consistent with data from sooty mangabeys demonstrating that vocal signals are specific to the social context in which they are produced (Range & Fischer, 2004). Moreover, the results from Gros-Louis (2002) provide evidence that communication serves to facilitate social interactions. However, that study contains a notable limitation. Specifically, infants produced significantly more trill vocalizations than did

any other age group (trills were produced infrequently by juveniles and adults). Therefore, this finding in infants could reflect an adaptation for alerting nearby caregivers of the infant's immediate needs and tells us very little about the social complexity and communicative behavior of adult white-faced capuchins. To make inferences about the origins of complex socio-communicative behavior, future studies should instead focus on a wider range of communicative signals and include individuals from multiple ages groups.

Consistent with these recommendations, Fan et al. (2018) studied the socio-communicative behavior of adult golden snub-nosed monkeys. Interestingly, the researchers found a positive association between social group size and vocal repertoire size, as well as between social structure – single male groups vs. multi-male groups – and vocal repertoire size (Fan et al., 2018). The authors concluded that these data support the social complexity hypothesis for communicative complexity. Specifically, the authors stated that the vocalizations of the golden snub-nosed monkey are related to distinct social roles, and therefore provide evidence for the hypothesis that vocalizations (and other communicative signals) evolved as a tool to navigate dynamic social environments (Fan et al., 2018). Whereas the findings are interesting, the researchers focused only on vocal repertoire size, and did not measure any other aspects of golden snub-nosed monkey communication.

A more comprehensive investigation of the relationship between social behavior and communication was conducted by Gustison, le Roux, and Bergman (2012) in gelada and chacma baboons. In this study, gelada baboons were considered to be the more socially complex species given gelada males form long-lasting relationships with a harem of females, while chacma males form only temporary, short-term bonds with females (Gustison et al., 2012). When comparing these baboon species, researchers found that gelada baboons had a significantly larger vocal

repertoire size than their less socially complex, chacma counterparts (Gustison et al., 2012). In addition to comparing gelada baboons and chacma baboons, the researchers also measured the frequency and social contexts of the vocalizations produced specifically by gelada baboons. Interestingly, the only derived calls (i.e., calls specific to gelada baboons) were those calls produced by males during social interactions with females (Gustison et al., 2012). Similarly, Guinea baboons employed a number of different vocalizations which correspond to the social contexts that they are produced in (Maciej, Ndao, Hammerschmidt, & Fischer, 2013). All told, these data provide further evidence that the primary function of communication is to regulate social interactions among conspecifics. For future researchers interested in the evolutionary origins of complex communicative systems in primates, Gustison and colleagues suggested shifting focus from species-level comparisons to individual-level socio-communicative behavior, as well as measuring the frequency of communicative production, rather than vocal repertoire size (Gustison et al., 2012; Maciej et al., 2013).

Whereas the results from the aforementioned studies in monkeys provide valuable evidence of a relationship between social behavior and communication, more elaborate examples exist in the great ape literature. In chimpanzees specifically, researchers have demonstrated the functional use of communication during aggressive interactions. For example, Slocombe and Zuberbühler (2007) found that during agonistic events, the screams produced by the receiving chimpanzee were reflective of the magnitude of aggression. This result suggests some chimpanzee vocalizations convey accurate information about social events. Additionally, the screams produced by victims of severely aggressive attacks were significantly exaggerated (as compared to the severity of the attack) if at least one higher-ranking chimpanzee was within close proximity to the attack (Slocombe & Zuberbühler, 2007). Furthermore, these data support

the hypothesis that communication functions to facilitate social interactions and navigate complex social environments and are consistent with findings in bonobos.

In comparison to chimpanzees and other nonhuman primates, bonobos have evolved a unique socio-sexual behavior thought to ease social tension (referred to as genital-genital rubbing; De Waal, 1995). This socio-sexual behavior is exhibited by all individuals and in all sex and age combinations (e.g., male-male, female-female, juvenile-adult, infant-infant, etc.). However, Clay and Zuberbühler (2012) focused on the use of copulation calls during female-female genital-genital rubbing only. The results from this study indicate copulation calls are associated with female social rank. Specifically, the researchers found that the proportion of genital-genital contacts that included a copulation call (vs. the proportion that did not include a call) was negatively corrected with female dominance rank; low-ranking females were more likely to produce a copulation call during genital-genital contacts than were high-ranking females (Clay & Zuberbühler, 2012). Additionally, low-ranking females were more likely to produce a copulation call during genital-genital contact if the other female was of a higher dominance rank than themselves. Furthermore, females were more likely to produce copulation calls during genital-genital contacts if a high-ranking female was within close proximity to the genital-genital rubbing event (Clay & Zuberbühler, 2012). Collectively, these results indicate that in bonobos, copulation calls evolved as a tool to navigate female-female social interactions. More broadly, these results suggest bonobos—like humans and chimpanzees—have complex cognitive abilities in terms of altering their socio-communicative behavior in response to changes in their social environment (e.g., understanding social roles and triadic relationships).

In conclusion, there is a burgeoning body of literature supporting the hypothesis that complex communication, and the cognitive capacities necessary for it, evolved as a tool to

maintain strong social bonds and to navigate dynamic social environments. Indeed, milestones in both social and communicative domains are necessary for typical socio-communicative development in humans, and nonhuman primates (Wobber et al., 2014). However, several limitations persist in the literature, including restricted study samples, the inclusion of simplistic or singular measures of behavior or communication, and a lack of focus on individual differences. Recently, researchers have suggested that there are shared biological factors influencing socio-communicative abilities in humans, bonobos, chimpanzees, and other mammals (Ebstein, Israel, Chew, Zhong, & Knafo, 2010; Johnson & Young, 2017; Staes, Bradley, Hopkins, & Sherwood, 2018). Therefore, it is imperative that researchers focus future investigations on individual differences in *multiple* aspects of socio-communicative behavior and consider the relative influence biological factors have on social behavior and cognition.

1.2 The Chronology of Social Cognition Development

In cases of typical development, human children selectively and preferentially attend to social stimuli, find social interactions intrinsically rewarding, and feel motivated to maintain social bonds (Chevallier, Kohls, Troiani, Brodkin, & Schultz, 2012; Durkin, 1995). It is interesting that several studies have demonstrated that many of the critical skill necessary for socio-communicative development emerge within the first year of life and are strongly associated with subsequent socio-communicative abilities (Markus, Mundy, Morales, Delgado, & Yale, 2000; Wagner, Luyster, Yim, Tager-Flusberg, & Nelson, 2013; Markus et al., 2000; Mitchell et al., 2006). This is particularly true for social orienting – selective and preferential attention to social (vs. nonsocial) stimuli in the environment. For example, Wagner and colleagues found that a child's level of visual attention to faces at 6 months of age is associated

with their communicative skills at 18 months of age (Wagner, Luyster, Yim, Tager-Flusberg, & Nelson, 2013). This finding along with work by Markus and colleagues and Mitchell and colleagues have led researchers to conclude that social orienting is a precursor to more complex cognitive processes, such as joint attention and theory of mind, and is necessary for typical socio-communicative development (Markus et al., 2000; Mitchell et al., 2006).

After social orienting emerges, typically developing infants begin to respond to behavioral requests by following the gaze and pointing gestures of other social agents – primarily their caregivers – by 4 to 6 months of age (Markus et al., 2000; Morales et al., 2000; Mundy, et al., 2007). This is referred to as receptive joint attention (RJA) and is thought to lay the foundation for later cognitive skills (Markus et al., 2000; Mundy et al., 2007). Specifically, Markus and colleagues found that responses to joint attention cues at 12 months were associated with language skills at 18 months (Mundy et al., 2007). Additionally, the authors indicated a need for further consideration of how receptive joint attention skills influence social and linguistic development.

A few months later, at the age of 9 to 12 months, typically developing children not only respond to behavioral requests, but they also begin to initiate them (Carpenter, Nagell, Tomasello, Butterworth, & Moore, 1998). This is referred to as initiating joint attention (IJA). Collectively, joint attention abilities have been linked to a variety of factors including theory of mind, social competence, and language proficiency (Vaughan Van Hecke et al., 2007; Wellman, Phillips, Dunphy-Lelii, & LaLonde, 2004). For example, Wellman and colleagues (2004) found that the latency to respond to joint attention cues predicted later theory of mind abilities in 14-month-old infants. Additionally, Delinicolas and Young, Iverson and Goldin-Meadow, as well as Morales and collaborators have shown that early joint-attention behaviors predict subsequent

language acquisition and communicative competence (Delinicolas & Young, 2007; Iverson & Goldin-Meadow, 2005; Morales et al., 2000). Delinicolas and Young (2007) concluded that joint attention impairments commonly associated with (ASD) are linked to aspects of language and social behavior. However, the authors stressed the need for additional investigations that can determine causal relationships between aspects of socio-communicative behavior and joint attention abilities.

A thorough investigation of the roles social orienting and joint attention play in socio-communicative development exists in literature on neurodevelopmental disorders, such as ASD. In cases of atypical development, deficits in the skills necessary for social orienting, joint attention, and communication are detectable at a very young age. Specifically, deficiencies in social orienting (selective attention to social stimuli) become detectable in children as young as 6 months old (Bhat, Galloway, & Landa, 2010; Chawarska, Macari, & Shic, 2013). For example, Chawarska, Maraci and Shic (2013) found that 6-month-old infants that were later diagnosed with ASD attended less often to social stimuli than infants that were not later diagnosed with ASD Additionally, Dawson and colleagues, as well as Mitchell and colleagues, have observed impairments in the response to, and production of, social and communicative cues in children as young as 9-12 months of age (Dawson et al., 2004; Mitchell et al., 2006). Furthermore, young children with ASD or at risk for developing ASD exhibit impaired abilities in both the initiation of joint attention and in responding to joint attention cues, as compared to typically developing children (Dawson et al., 2004; Loveland & Landry, 1986). Similarly, in their 2006 study, Toth, Munson, Meltzoff and Dawson found that the initiation of and response to joint attention cues in infancy are strongly associated with later language abilities in children diagnosed with ASD (Toth, Munson, Meltzoff, & Dawson, 2006).

An additional precursor to typical social and cognitive development in humans is early social interest. Indeed, Adamson, Deckner, and Bakeman (2010) found that children diagnosed with ASD lost interest in social stimuli rapidly and were more interested in images of objects than images of people. This lower social interest was associated with poorer joint attention abilities and prolonged development of symbol-based language acquisition (Adamson, Deckner, & Bakeman, 2010). In addition, Adamson, Bakeman, Suma, and Robins (2019) found evidence that infants at risk of developing ASD, including those that are later diagnosed with ASD, have poorer joint attention abilities and expressive language. Furthermore, in both developmentally delayed children and children later diagnosed with ASD, joint engagement at 24 months predicted expressive vocabulary at 31 months (Adamson, Bakeman, Suma, & Robins, 2019). Collectively, these findings suggest that early social attention and interest lay the foundation for subsequent behavioral and linguistic development. It is surprising, however, that the researchers focused on children at 24 months of age, when social orienting and attentional deficits can emerge as young as 6 months old.

Indeed, two major limitation of previous studies include conducting investigations in children whom are past the critical developmental period, and a focus on higher-order cognitive processes, such as theory of mind (Chevallier et al., 2012; Fletcher-Watson et al., 2014; Zwaigenbaum et al., 2015). The specific criticism is that children may have already developed notable impairments in various foundational skills, such as social orienting and joint attention, by the time theory of mind and other cognitive capacities emerge. Given that more advanced cognitive skills such as theory of mind, do not develop until much later in life (4 to 5 years of age), researchers have recently shifted focus to more foundational processes that occur earlier in development (Jones, Dawson, Kelly, Estes, & Webb, 2017; Murza, Schwartz, Hahs-Vaughn, &

Nye, 2016; Zwaigenbaum et al., 2015). Indeed, successful interventions aimed at improving socio-communicative abilities are implemented as early as possible and typically involve long-term social skills training (Camargo et al., 2016; Jones et al., 2017; Zwaigenbaum et al., 2015).

Unfortunately, the results from cognitive assessments and intervention studies aimed at improving socio-communicative abilities have been mixed (Chevallier et al., 2012; Fletcher-Watson, McConnell, Manola, & McConachie, 2014; Gates, Kang, & Lerner, 2017; Shepherd, Landon, & Goedeke, 2018). Researchers suggest that this may be due to a number of factors including a focus on advanced cognitive processes, a lack of control over extraneous variables, limited consistency in intervention procedures, and an inability to examine individual differences (Ames & Fletcher-Watson, 2010; Gates et al., 2017; Reichow, 2012; Wolstencroft et al., 2018; Zwaigenbaum et al., 2015). All told, social orienting and joint attention skills form the foundation for higher-order cognitive abilities and language proficiencies and are therefore critical for typical socio-communicative development (Delinicolas & Young, 2007; Mitchell et al., 2006; Vaughan Van Hecke et al., 2007).

1.3 From Genotype to Phenotype

To better illustrate how biological factors influence individual-level socio-communicative behavior and social cognition, this review will highlight a single genetic factor and its potential pathway for influencing the behavioral phenotype associated with socio-communicative disorders. Specifically, one potential biological factor underlying individual differences in socio-communicative behavior and social cognition is the arginine vasopressin receptor gene 1A (*AVPR1A*) (Bachner-Melman et al., 2005; Donaldson et al., 2008; Staes et al., 2018). Variations in *AVPR1A* have been linked to a number of socio-communicative behaviors in humans and

nonhuman mammals, making it a key gene of interest (Johnson & Young, 2017; Mahovetz, Young, & Hopkins, 2016; Staes et al., 2016; Wang et al., 2016). Indeed, researchers across multiple disciplines have demonstrated the critical role genes like *AVPR1A* play in social behavior, communication, and social cognition in humans and nonhuman great apes (Bachner-Melman et al., 2005; Donaldson et al., 2008; Staes et al., 2018; Muholland et al., 2020).

Much of what we know about *AVPR1A* and its influence on various socio-communicative processes comes from foundational work in rodents. For example, Beilsky and colleagues were interested in how knocking out *AVPR1A* would impact social behavior and social cognition in a mouse model. Specifically, they found that knock-out mice for *AVPR1A* spent significantly less time interacting with social partners than did wild-type mice and lacked any individual social memory (Bielsky, Hu, Szegda, Westphal, & Young, 2004; Egashira et al., 2007). Additionally, Pitkow and colleagues, as well as Winslow and colleagues demonstrated that increasing AVP receptor expression facilities pair-bonding and induces partner preference in male voles (Pitkow et al., 2001; Winslow, Hastings, Carter, Harbaugh, & Insel, 1993). Similarly, Young and colleagues determined that transgenic mice carrying the AVP receptor of monogamous prairie voles, exhibited increases in social interactions following AVP injection (Young, Nilsen, Waymire, MacGregor, & Insel, 1999). Unfortunately, studies in rodents are considered to be limited due to fact that rodents do not exhibit particularly complex socio-communicative behavior (Bauman & Schumann, 2018; Putnam, Young, & Gothard, 2018). More recently, investigators have studied the influence of *AVPR1A* sequence variation on socio-communicative behaviors in the species most closely related to humans.

Over the course of hominid (orangutans, gorillas, chimpanzees, bonobos and humans) evolution, there is evidence indicating a tandem duplication of a non-coding, 5’ flanking region

of *AVPR1A* (DupB) (Donaldson et al., 2008). The DupB region (~350BP) contains a complex (CT)4-(TT)- (CT)8-(GT)24 polymorphic motif, known as the RS3 microsatellite (Donaldson et al., 2008). In their 2008 study, Donaldson and colleagues found that among hominids, chimpanzees are unique in that they are polymorphic for a secondary deletion of the DupB microsatellite-containing element in the 5' flanking region of *AVPR1A* (Donaldson et al., 2008). Additionally, preliminary data in chimpanzees indicate that individuals without the RS3 duplication (DupB-) spend less time in close proximity to conspecifics (Evans, 2016). Similarly, Hopkins and colleagues found evidence that chimpanzees with a DupB- genotype (e.g., shorter *AVPR1A* allele length) perform significantly poorer on social cognition tasks and responded less to socio-communicative cues than chimpanzees with a DubB+ genotype (e.g., a longer *AVPR1A* allele length (Hopkins et al., 2014; Mahovetz et al., 2016).

In contrast to chimpanzees, bonobos and humans have both copies of the microsatellite (DupB+) and, instead of a species-wide polymorphism, individuals exhibit variations in the allele length of the RS3 microsatellite (Donaldson et al., 2008). In humans, allelic variations in *AVPR1A* have been associated with social behavior and pair-bonding, as well as individual differences in aspects of social cognition (Oztan et al., 2018; Wade, Hoffmann, & Jenkins, 2014; Walum et al., 2008; Wang et al., 2016). Specifically, individuals with a shorter RS3 microsatellite were less likely to act altruistically and reported higher levels of social conflict with their siblings, than did individuals with a longer RS3 microsatellite length (Bachner-Melman et al., 2005; Knafo et al., 2008). Additionally, Walum and colleagues (2008) demonstrated that males with a shorter genotype reported lower levels of partner bonding than males with a longer genotype. More recent results indicate males with a longer *AVPR1A* genotype perform more altruistically than males with a shorter *AVPR1A* genotype (Wang et al.,

2016). In addition, Zhang and colleagues (2020) found that humans with a shorter *AVPR1A* allele length had poorer verbal memory than humans with a longer *AVPR1A* variant. It is interesting that data from children are consistent with these findings. For example, Avinun and colleagues investigated the relationship between performance on social cognition tasks and *AVPR1A* variation. Specifically, they found that preschoolers with a shorter *AVPR1A* allele length performed worse on a social cognition task measuring altruistic behaviors than those with a longer allele length (Avinun et al., 2011).

In addition to behavioral studies, biological studies provide a considerable amount of support for *AVPR1A*'s influence on socio-communicative processes. Specifically, Tansley and colleagues (2011) found that gene reporter assays conducted in human cell lines indicate shorter *AVPR1A* microsatellite genotypes are associated with lower *AVPR1A* transcription. Similarly, Meyer-Lindenberg et al. (2009) found that humans with a shorter *AVPR1A* allele length exhibited lower neural activity in the amygdala during a face recognition tasks than did individuals with a longer allele length. Additionally, results from transmission disequilibrium tests suggest individuals with a shorter *AVPR1A* genotype (as compared to individuals with a longer genotype) may be more susceptible to develop behavioral and cognitive impairments such as those associated with Autism Spectrum Disorder (ASD) and other socio-communicative disorders (Kim et al., 2002; Wassink et al., 2004; Yirmiya et al., 2006). Indeed, Oztan and colleagues determined that lower *AVPR1A* gene expression predicted greater social impairments and greater stereotypical behaviors in children diagnosed with ASD, as compared to higher *AVPR1A* gene expression in their 2018 investigation (Oztan et al., 2018).

Collectively, these studies suggest a predictive relationship between *AVPR1A* genotype and individual socio-communicative abilities. Specifically, shorter RS3 microsatellite allele

length may predispose individuals to have lower neurobiological responses during social information processing. In turn, this may lead to poorer social orienting skills, reduced communicative production, and ultimately notable behavioral and cognitive impairments like those associated with ASD (e.g., impaired joint attention abilities, language difficulties, repetitive stereotypical behaviors, etc.; Oztan et al., 2018; Yang et al., 2017; Zhang et al., 2020). Despite this association between *AVPR1A* and social behavior in humans, it remains unclear whether *AVPR1A* genotype is predictive of the ASD phenotype in children. If genetic variants, such as the *AVPR1A* RS3 microsatellite, are in fact predisposing individuals to develop socio-communicative impairments, like those associated with ASD, then it is critical to include objective behavioral and cognitive measures. Unfortunately, previous studies with humans and nonhuman animals have typically focused on subjective, indirect assessments of behavior or on single measures of behavior *or* cognition (See Table 1.3). For future projects, researchers stated that it is imperative to focus on individual differences and include comprehensive measures of behavior, communication, and cognition (Knafo et al., 2008; Oztan et al., 2018; Wang et al., 2016).

Table 1.3 Studies in which AVPR1A's Influence on Social Behavior, Communication, and Social Cognition were Investigated

Article	**Species**	**Behavioral/Cognitive Measure(s)**	**Primary Finding**
Staes et al. (2016)	Bonobo	Personality questionnaire scores	Individuals with long/long genotype had higher attentiveness scores and lower openness scores than individuals with long/short genotype
Evans (2016)	Bonobo & Chimpanzee	Social proximity (nearest neighbor measure) from observations	Dup- chimpanzees were more likely to spend time alone than Dup+ chimpanzees and bonobos
Hopkins et al. (2014)	Chimpanzee	Receptive joint attention	Dup+ males needed less socio-communicative cues to respond to a behavioral request than did Dup- males

Mahovetz et al. (2016)	Chimpanzee	Mirror self-recognition	Dup+ males had higher rates of scratching than Dup- males, Dup+ females, and Dup- females
Staes et al. (2015)	Chimpanzee	Personality traits derived from observations	*AVPR1A* genotype predicted sociability scores for males and females
Avinun et al. (2011)	Human	Modified Dictator's Game – Altruism	*AVPR1A* RS3 microsatellite variation is associated with funds allocations in preschool-age children
Bachner-Melman et al. (2005)	Human	Conflict scale from sibling relationship questionnaire	Significant linkage between *AVPR1A* genotype and self-reported sibling conflict scores
Knafo et al. (2008)	Human	Dictator Game - Altruism	Individuals with a short genotype allocated less funds (acted less altruistically) than did individuals with a long genotype
Walum et al. (2008)	Human	Self-reported partner bonding scales	Men with a longer genotype reported higher scores for pair-bonding than did men with a shorter genotype
Wang et al. (2016)	Human	Dictator Game - Altruism	Males with a shorter genotype allocated less funds (acted less altruistically) than did males with a longer genotype
Oztan et al. (2018)	Human	Questionnaires for social impairments and stereotypical behaviors	Lower AVP receptor gene expression (as compared to higher) predicted greater social impairments and greater stereotypical behaviors in children. Lower AVP receptor gene expression predicted disease status (likelihood that the child is diagnosed with autism spectrum disorder)
Zhang et al. (2020)	Human	Verbal memory performance scores	Individuals with a shorter *AVPR1A* genotype had poorer verbal memory skills than those with a longer genotype

2 BACKGROUND AND SIGNIFICANCE

2.1 Socio-Communicative Behavior in Great Apes

To understand better how genetic, behavioral and cognitive factors contribute to the emergence of typical and atypical socio-communicative development in humans, a burgeoning body of literature highlights the unique value of studying these mechanisms in *nonhuman* animals, especially those most closely related to humans (Donaldson et al., 2008; Hammock & Young, 2006; Staes et al., 2018). The family *Hominidae* includes modern humans, bonobos, chimpanzees, gorillas, and orangutans. Of all of the nonhuman great ape species, the two most closely related to humans are chimpanzees (*Pan troglodytes*) and bonobos (*Pan paniscus*)– diverging from a common ancestor ~6 million years ago (Jensen-Seaman, Deinard, & Kidd, 2001; Prufer, Munch, Hellmann, Akagi, Miller, et al., 2012; Rogers, 1993).

It is interesting that bonobos and chimpanzees exhibit complex socio-communicative behavior, have the largest foraging party sizes and vocal repertoire sizes of all nonhuman apes, and have the highest rates of grooming among all ape species (Stanford, 1998; Harcourt, Hauser, & Steward, 1993; Mackinnon, 1974; McComb & Semple, 2005). Both species live in highly social, multi-male, multi-female groups, and use socio-communicative behaviors to influence overall social standing (Liebal et al., 2014; Gruber & Clay, 2016). Additionally, bonobos and chimpanzees demonstrate the most flexibility in the production and perception of communicative signals out of all of the nonhuman great ape species and modify their communication depending on social context (Clay & Zuberbühler, 2012; Gruber & Clay, 2016; McComb & Semple, 2005; Slocombe & Zuberbühler, 2007). Specifically, chimpanzee males scream louder during aggressive acts if an alpha male is nearby than if an alpha male is not in proximity (Slocombe & Zuberbühler, 2007). In addition, subordinate bonobo females scream louder when they are

genital-genital rubbing with a female that is of higher rank, as compared to a female that is of equal or lower rank than themselves (Clay & Zuberbühler, 2012). Furthermore, bonobos and chimpanzees are also the only nonhuman great ape species to live in fission-fusion societies.

In addition to exhibiting complex socio-communicative behavior, there is evidence that chimpanzees and bonobos (and many other nonhuman primates) engage in repetitive abnormal/stereotypical behaviors (Bloomsmith, Clay, Lambeth, Lutz, Breaux, et al., 2019; Brand, Boose, Squires, Marchant, White, et al., 2016; Lameris et al., 2021). Specifically, captive chimpanzees and bonobos have been observed to engage in rocking, coprophagia (i.e., consuming feces), hair plucking, as well as self-directed and self-injurious behaviors (Bloomsmith, et al., 2019; Brand, et al., 2016; Lameris et al., 2020). Furthermore, self-directed hair plucking is associated with elevated urinary cortisol levels in female bonobos, suggesting that repetitive/stereotypical behaviors are linked to stress and welfare in the species most closely related to humans (Brand, et al., 2016).

Despite these behavioral similarities and the fact that chimpanzees and bonobos only diverged from a common ancestor with each other approximately 1.5 million years ago, the two species display noted differences in social structure, vocal flexibility, and feeding ecology (Jensen-Seaman, Deinard, & Kidd, 2001; Prado-Martinez, Sudmant, Kidd, Li, Kelley, et al., 2013; Prufer, Munch, Hellmann, Akagi, Miller, et al., 2012; Rogers, 1993). Bonobos are matriarchal, regarded as more tolerant and egalitarian than their chimpanzee counterparts, and participate frequently in socio-sexual behavior (Parish, de Waal & Haig, 2000; Stanford, 1998; Hare, Melis, Woods, Hastings, & Wrangham, 2007). On the contrary, chimpanzees are considered to be a more belligerent and territorial species that participates in higher levels of intra- and inter-specific aggression (de Waal, 2007; Stanford, 1998). Additionally, bonobos

travel in larger feeding parties, live in larger social groups, and have larger vocal repertoires that they utilize more flexibly as compared to chimpanzees (Doran, Sugiyama, Fleagle & Heesy, 2002; Stanford, 1998; Moore, 2014; McComb & Semple, 2005; Bermejo & Omedes, 1999; Rowe, 1996). Collectively, these findings have led to the common perspective that bonobos are more social than chimpanzees. Indeed, preliminary data indicate bonobos spend more time engaged with social partners and communicate ~3x more often than chimpanzees (Skiba, 2017).

Surprisingly, no study has assessed the relationship between social behavior, communicative production, and repetitive/abnormal behaviors within the same sample of great apes. A better understanding of the relation between social behavior and communication in bonobos could provide insight into how human social behavior and language co-evolved – resulting in the most complex socio-communicative system in the animal kingdom. Therefore, I aimed to examine the rich and complex social behaviors of bonobos in a captive setting through behavioral observations, while also recording the frequency of communicative production. Given how important communication is for navigating complex social environments, *I predicted that individual-level social behavior would be positively associated with communicative production. Additionally, given the prevalence of repetitive/abnormal behaviors in children diagnosed with ASD, I predicted that individual-level social behavior and communicative production would be negatively associated with repetitive/stereotypical behaviors.*

2.2 Social Cognition in Great Apes

Like humans, bonobos and chimpanzees produce a variety of complex communicative signals (e.g., vocalizations, gestures, facial expressions, and multisource signals), and can flexibly employ these signals and modify them depending on the social context (Clay, Pika,

Gruber, & Zuberbühler, 2011; Gruber & Clay, 2016; Laporte & Zuberbühler, 2010; Meltzoff & Moore, 1977). Additionally, researchers have demonstrated that bonobos and chimpanzees are capable of learning and utilizing an arbitrary symbol-based language system (Savage-Rumbaugh, Rumbaugh, & Boysen, 1978; Savage-Rumbaugh, Murphy, Sevcik, Brakke, Williams, et al., 1993; Rumbaugh, 1977). Not surprisingly, much of the research done on social cognition in nonhuman animals has been conducted with bonobos and chimpanzees.

It is interesting that individuals from both *Pan* species are sensitive to the visual attentiveness of a human social partner and modify their behavior depending on the human's attentional state (Lucca, MacLean, & Hare, 2017). Similarly, bonobos and chimpanzees spend more time looking at images of known conspecifics than images of unknown conspecifics, suggesting that social factors influence social attention in the species most closely related to humans (Lewis, Kano, Stevens, DuBois, Call, & Krupenye, 2021). Additionally, individuals from both species engage in inter and intra-specific joint attention (Pitman, & Shumaker, 2009; Leavens, & Racine, 2009). Specifically, there is evidence to suggest that both chimpanzees and bonobos are sensitive to and can follow the head movements and eye-gaze of a human experimenter (Tomasello, Hare, Lehmann, & Call, 2007) In addition, researchers have documented that joint attention abilities are linked to imitative learning (a higher-order cognitive ability) in chimpanzees (Carpenter & Tomasello, 1995).

Much like in the case of humans, aspects of socio-communicative behavior and social cognition have been associated with neurobiological and genetic variation in chimpanzees and bonobos (Hopkins, Misura, Reamer, Schaeffer, Mareno, et al., 2014; Hopkins, Keebaugh, Reamer, Schaeffer, Schapiro, & Young, 2014; Staes, et al., 2015). Specifically, receptive joint attention abilities have been linked to anatomical differences in the superior temporal gyrus

(responsible for multisensory integration and the site of Wernicke's area in humans) of chimpanzees, as well as to polymorphisms in *AVPR1A* (Hopkins, et al., 2014a; Hopkins, et al., 2014b). For example, DupB+ male chimpanzees performed significantly better on a receptive joint attention task and were significantly more responsive to the human experimenter's socio-communicative cues than chimpanzee males with the DupB- variant (Hopkins, et al, 2014a). Similarly, preliminary data in chimpanzees suggest that individuals that produce attention-getting vocalizations have enhanced local connectivity in the inferior frontal gyrus (responsible for processing speech and language and the site of Broca's area), as compared to chimpanzees that do not produce these vocalizations (Skiba, Hopkins, & Taglialatela, 2016). Together, these findings suggest that similar neurobiological and genetic mechanisms may underlie socio-communicative behavior and social cognition in bonobos, chimpanzees, and humans.

Unfortunately, little is known about the relation between overt socio-communicative behavior and functional social cognition in humans and nonhuman great apes. This may in part be due to the fact that many investigators have only included a single measure of behavior or cognition (e.g., social interest, receptive joint attention, grooming rates) or subjective measures of socio-communicative behavior (e.g., keeper or guardian questionnaires, personality assessments, and social/dominance rank) in their studies (Hopkins, et al., 2014a; Lewis, et al., 2021; Oztan, et al., 2018; Rodrigues & Boeving, 2019; Staes, et al., 2015). This may in part be due to the ease of collecting singular, subjective measures in captive primates. It is certainly challenging to collect multiple objective measures of behavior and cognition in captive bonobos or chimpanzees within the same study. However, this does not negate or minimize the importance of studying the relation between observable socio-communicative behavior and functional social cognition in the species most closely related to humans.

Therefore, I aimed to determine whether or not individual-level socio-communicative behavior or stereotypical behavior was associated with performance on initiating and receptive joint attention tasks in captive bonobos. To this end, I collected individual-level performance data on joint attention tasks. Given joint attention is the first developmental milestone that incorporates both social and communicative components, *I predicted individual levels of social behavior and communication would be positively associated with performance on joint attention task.* Additionally, *I predicted individual level of stereotypical behavior would be negatively associated with performance on joint attention tasks.*

2.3 Genetic Basis of Socio-Communicative Development in Great Apes

Given the social and communicative deficits associated with ASD can greatly impair daily functioning and persist into adulthood, it is critical to distinguish potential biological factors that may aid in identifying children at risk of developing socio-communicative disorders. Indeed, the Simons Foundation has created a database of genes associated with aspects of the ASD behavioral phenotype – SFARI Gene (https://gene.sfari.org/). Of particular interest are genes with an association score of 1 or 2, reflecting strong empirical evidence for an association - implicated by a genome-wide association study or consistently replicated and accompanied by evidence that the risk variant has a functional effect (Abrahams, Arking, Campbell, Mefford, Morrow, et al., 2013).

Three genes with variants that are highly associated with the ASD behavioral phenotype and present in the bonobo genome, include arginine vasopressin receptor gene 1A (*AVPR1A*), oxytocin receptor (*OXTR*), and forkhead box protein 2 (*FOXP2*). *AVPR1A* codes for vasopressin receptors in the brain and has been linked to a number of social behaviors in humans and

nonhuman apes (Donaldson et al., 2008; Oztan et al., 2018; Wade, Hoffmann, & Jenkins, 2014; Walum et al., 2008; Wang et al., 2016). Variants of *AVPR1A* have also been associated with aspects of the ASD behavioral phenotype (Kim et al., 2002; Wassink et al., 2004; Yirmiya et al., 2006). Similarly, several studies have found that *OXTR* plays an important role in social bond formation and social motivation (Wu & Su, 2018; Theofanopoulou et al., 2020). Specifically, researchers have documented that *OXTR* genetic variation is related to social affiliation, vocal symptoms, and socio-communicative impairments associated with ASD (Wermter, et al., 2010; Oztan, et al., 2018; Holmqvist Jamsen, et. al., 2017). It is interesting that *FOXP2* is one of the first genes to be associated with human language disorders and fine orofacial motor control, as two functional copies are required for normal development of communication in great apes, and speech and language in humans (Staes, et al., 2017; Fisher & Scharff, 2009). Polymorphisms found in *FOXP2* affect the regulations of protein expression, leading to differences in socio-communicative behavior (Mozzi, Riva, Forni, Sironi, Maroni, et al., 2017).

More recently, single nucleotide polymorphisms (SNPs) of *OXTR*, *AVPR1A*, and *FOXP2* have all been associated with differences in socio-communicative behavior, as well as with aspects of the ASD behavioral phenotype (Gong et al, 2004; Mozzi et al., 2017; Tansey et al., 2011; Watanabe et al., 2017; Zhang et al., 2018). For *OXTR*, SNPs have been associated with social abilities in typical populations, as well as with increased severity in social deficits associated with ASD (Baribeau, et al., 2017). Additionally, there is considerable evidence for the influence of SNPs in *AVPR1A* and *OXTR* on empathy, prosocial temperament, social sensitivity, and stress reactivity in social contexts in individuals diagnosed with ASD (Cataldo et al, 2018). Collectively, these studies suggest that SNPs in *AVPR1A*, *OXTR*, and *FOXP2* influence socio-communicative abilities and

are a critical area of research for neurodevelopmental disorders. Interestingly, variation in *AVPR1A*, *OXTR*, and *FOXP2* have also been linked to aspects of social behavior and social cognition in great apes (Hopkins et al, 2012; Staes et al., 2014; Staes et al, 2017; Weiss et al, 2021). Unfortunately, previous studies in nonhuman apes have focused on indirect assessments of behavior or single measures of behavior or cognition (Table 1.3). Indeed, the current study is the first comprehensive assessment of individual-level social behavior, communication, repetitive/stereotypical behavior, and joint attention abilities in within a single species of great ape. Therefore, I aimed to determine whether or not any of the SNPs in *AVPR1A*, *OXTR*, and/or *FOXP2* that have been implicated in ASD in humans are also polymorphic bonobos. Additionally, I aimed to determine whether or not these specific SNPs in bonobos predict individual levels of social behavior, communicative production, and stereotypical behavior, *as well as* performance on social cognition tasks in bonobos**.** Given the roles of *AVPR1A*, *OXTR*, and *FOXP2* in social behavior and cognition in humans and other apes, and the association between SNPs in these genes and aspects of the behavioral phenotype of ASD, *I hypothesized that SNPs in AVPR1A, OXTR, and/or FOXP2 would also be polymorphic in bonobos. Additionally, I hypothesized that SNP variant would predict how social and communicative an individual is, the number of repetitive/stereotypical behaviors they produce, as well as their joint attention abilities.* All told, the data generated from this study fill a critical gap in our understanding of how genetic factors influence typical and atypical socio-communicative development. Furthermore, the results of this study could identify specific SNPs in *AVPR1A*, *OXTR*, and/or *FOXP2* as promising biological factor underlying socio-communicative abilities in bonobos, supporting the notion that bonobos are an ideal model for socio-communicative disorders in humans.

3 METHODS

3.1 Overview and Study Design

Subjects included 26 bonobos housed at the Ape Initiative (n=7), the Milwaukee County Zoo (n=13), and the Columbus Zoo and Aquarium (n=7). Behavioral observation data were collected from 26 subjects, and joint attention assessments were conducted on the 7 individuals

from Ape Initiative. For the genetic analyses, whole blood samples (n=6) and buccal swabs were collected (n=23). It is important to note that this project does not include behavioral data on all of the individuals that biological samples were collected from. Additionally, one subject, a female adult from Milwaukee County Zoo, was excluded from all analyses given her infant offspring was removed from her social group halfway through data collection.

Table 3.1 Subjects Table

Facility	Subjects	Male:Female	Age Range
Ape Initiative	**7**	**4:3**	**10 – 40**
Milwaukee County Zoo	**12**	**5:7**	**9 – 53**
Columbus Zoo & Aquarium	**7**	**3:4**	**8 – 35**
Total	**26**	**12:14**	**8 – 53**

3.2 Behavioral Data Collection

To quantify socio-communicative behavior, eight 10-minute observations (i.e., focal follows – observing the behavior of a single individual) were collected from each subject. Specifically, I was interested in social proximity (the physical distance to the nearest social partner), social proportion (the proportion of time spent engaged in the social behaviors of grooming and play), communicative production, and stereotypical/repetitive behaviors (see descriptions of these behavioral measures below in Table 3.2). Focal follows were collected between the hours of 08:00 and 18:00 while the bonobos were in their typical home enclosures. The order of focal follow observation sessions was randomized each day by assigning a number to each subject alphabetically and then selecting a random number before starting each observation session. For each subject, only one 10-minute observation was collected in a 60-

minute time window, and no more than three 10-minute observations were collected in a single day. If the focal individual went out of view for more than three minutes, or their proximity could not be determined for three or more data points, the observation was discontinued and not used in data analyses. All observations were also audio/video recorded using a Canon VIXIA HF G30 camcorder. Sociality and communicative production measures were coded in real-time by the experimenter during the audio/video recording. Once all focal observations were collected, audio/video files were coded post hoc using Behavioral Observation Research Interactive Software (BORIS) version 7.10.5 operating on a MacBook Air. Social proximity was recorded instantaneously at 1-minute intervals (starting at time zero) for a total of 11 proximity data points per observation (see proximity measures below). All other social behavior measures were recorded continuously during the 10-minute focal observation (see state behaviors below) or on an all-occurrence basis (see event behaviors below). Social group sizes varied across facilities and across data collection days and were recorded for every observation.

Any communicative signal directed towards an external source (e.g., a human caregiver) was noted during data collection and included as a separate variable for all analyses (external signals). Signals were coded as external if the signaler emitted the communicative signal while either 1) performing a focused look or glance towards a human, or 2) approaching a human. All communicative signals (any signal produced by the focal individual not directed towards humans) were coded as either a vocalization, gesture, facial expression, or co-occurring signal (see Table 3.2) When a specific signal type was necessary for the other signal's production it was coded as the primary signal type, and if it was not necessary for the other signal's production, it was coded as a co-occurring signal. For example, a 'hoo' vocalization that contains a 'hoo' face was coded as a vocalization because the facial expression was necessary to produce the 'hoo'

sound. In contrast, a play face is not necessary for the production of a laugh vocalization. Therefore, if the focal produced a play face while laughing, the event was coded as co-occurring. A communicative event was considered a separate signal if it occurred at least 3 seconds following the production of the previous signal or was of a different type and did not fit the co-occurring signal criteria. For example, if an individual vocalized at the beginning of an agonistic event, and 2 seconds later aggressively hit the conspecific, these communicative events were coded as two distinct communicative signals (vocalization and gesture), given they are of distinct signal types and do not fit the co-occurring criteria.

During each 10-minute focal follow, I also recorded the frequency of repetitive/stereotypical behaviors. Specifically, I recorded repetitive motor movements (e.g., hand-flapping, knuckle-rubbing, rocking), abnormal behaviors (e.g., urine drinking, coprophagia, regurgitation and reingestion) and self-directed behaviors (e.g., head-banging, self-biting, hair-plucking).

Table 3.2 Socio-Communicative Measures

State Behaviors	**Description**
Groom Give (GG)	Focal individual initiated and is grooming social individual without any grooming returned.
Groom Receive (GR)	Focal individual is receiving grooming initiated by another social individual and is not returning any grooming.
Groom Mutual (GM)	Both the focal individual and the social partner(s) are actively grooming each other –grooming partner.
Play (PL)	Focal individual engages in lively activity with another individual by wrestling, tickling and/or chasing the conspecific.
Nursing Give (NG)	Focal individual supplies a conspecific (usually offspring) with milk while conspecific is attached to focal individual's nipple.
Nursing Receive (NR)	Focal individual suckles on nipple of conspecific female (usually mother) to receive milk.

Event Behaviors	**Description**

Genital - Genital Rubbing / Copulation (GG)	Focal individual is actively engaged in sex or genital-genital contact with a conspecific.
Displaying (DS)	Focal individual is swaying, charging, drumming, slapping the ground/walls/barrels, or throwing objects; accompanied by pilo-erection.
Communicative Signal (CS)	Any vocal, gestural, facial or concomitant signal produced by the focal individual. In order to be considered a separate/new signal, the signal must be produced at least three seconds following a prior signal or must be of different signal type than the prior signal. See communicative signal types below.
Aggression Give (AG)	Focal individual is slapping, biting, hitting, and/or chasing a conspecific, while pilo-erect.
Aggression Receive (AR)	Focal individual is being slapped, bitten, hit, and/or chased by a pilo-erect conspecific.
Aggression Mutual (AM)	Both the focal individual and the social partner(s) are actively slapping, biting, hitting, or chasing each other, while pilo-erect.
Repetitive/Stereotypical Behavior (RB)	Focal individual engages in a repetitive motor movement (e.g., hand-flapping, knuckle-rubbing, rocking), abnormal behavior (e.g., urine drinking, coprophagia, regurgitation and reingestion) or self-directed behaviors (e.g., head-banging, self-biting, hair-plucking)

Social Proximity	**Description**
Close/Touching (TOU)	Focal individual is in physical contact with a conspecific or close enough that it could touch a conspecific without relocating (≤ 1.5 meters).
Socially Close (CLO)	Focal individual is ~ 1.5-3 meters from the nearest conspecific.
Solitary (SOL)	Focal individual is ~ 3-5 meters from the nearest conspecific.
Isolated (ISO)	Focal individual is > 5 meters from the nearest conspecific.

Communicative Signals	**Description**
Vocalization (VO)	Any vocal signal produced by focal individual. See vocal types and definitions below. This includes idiosyncratic sounds produced during manual grooming by chimpanzees.
Gesture (GE)	Any gestural signal produced by focal individual. See gestural types and definitions below.
Facial Expression (FA)	Any facial communicative signal produced by focal individual that is not produced in conjunction with a vocalization that may necessitate it. (For example 'hoo' faces produced without a 'hoo' vocalization are considered facial expression, while 'hoo' vocalizations with a 'hoo' face are considered vocalizations, not concomitant signals). See facial expression types below.

Multi-Source Signal (MS)	Any vocalization, gesture, facial expression or body posture that occurs within two seconds of an initial communicative signal of different type. (VO+GE, GE+FA, VO+FA)

Bonobo Vocal Types

Vocalization Types	**Description**
Hoots (HO)	Relatively loud vocalizations that are produced in a series and are voiced on both inhalation and exhalation. Hoots are often produced by a number of individuals simultaneously.
Alarms (AL)	Loud, sharp vocalizations that may sound like a "wraa" or "waa". Alarms are given in the context of real or perceived danger (snake, truck, etc.).
Peeps/Peep Yelps (PE)	Generally short, tonal, high-pitched vocalizations that are produced in a variety of contexts. They may occur in series or as a single call. They may be modulated or not (i.e. no change in frequency).
Pants/Grunts (PG)	Relatively low frequency, noisy vocalizations that are usually produced in a series. Pants are fast, repetitive, low frequency vocalizations made on both inhalation and exhalation (e.g. panting laugh). Grunts are also relatively quiet (but louder than pants) and sound like a series of "ohoh" or "uhuh" sounds made in quick succession.
Screams (SC)	Very loud, high pitched, relatively long vocalizations that have both tonal and noisy components.
Copulation Scream (CS)	Much like that of regular screams but higher pitched and produced during copulation or g-g rubbing.
Other (OT)	A vocalization that does not meet any of the above requirements for a category should be classified as other.

Bonobo Gesture Types

Gesture Types	**Description**
Food Beg (FB)	Focal individual extends arm towards another individual with palm facing up and hand maintained in a cupped posture. May include placing one or both hands around or under the other's lips and or chin.
Wrist/Finger Present (WP)	Focal individual flexes the wrist while holding the back or side of hand out toward another individual, may include placing a finger or hand into another individual's mouth. Note whether or not contact occurs.
Point (PO)	Focal individual directs either his/her whole hand or one or more digits to recipient, another individual or object in the environment. Subject holds out a hand toward another individual or object by extending the arm, wrist, and hand. May end in contact but gesture initiates without contact.

Touch (TO)	Focal individual makes any sort of contact with another individual with the front or back of their hand or fingers. Common Touch Gestures: GT=Gentle touch, DB=Dab, PT=Pat, EM=Embrace, AH=Aggressive hit, GR=Grab and GI=Genital inspect (with hand), PK=Poke.
Threat Gesture (TG)	Focal individual swings arm in a quick, upward motion towards another individual with palm facing down (AR=Arm raise) or shakes the hand vigorously and repeatedly with a flexible wrist towards another individual (WS=Wrist shake).
Other (OT)	Focal individual produces a gesture that does not fall into one of the other categories. Common Other Gestures: AO=Arm over, CB=Cage bang, TH=Throw, CL=Clap, SH=Self hit, RK=Rap knuckles

Bonobo Gesture– Sub Types

Touch Gestures	**Description**
Aggressive Hit (AH)	Individual uses hand to strike recipient with force, pilo-erect.
Dab (DB)	Subject touches recipient with back of flexed fingers whereafter touching hand is withdrawn immediately; sequence is repeated in quick succession.
Embrace (EM)	Individual places one or two arms around another, generally around their back from the front but can be from the back around their middle.
Genital Inspect (GI)	Subject touches recipient's swelling or penis with fingertip(s) or hand.
Grab (GR)	Subject uses his/her hand(s) to forcefully grasp recipient.
Gentle Touch (GT)	Subject makes any sort of contact with another individual with the front or back of their hand or fingers, without appreciable force, that does not fall into one of the more specific categories of touch.
Poke (PO)	Subject pushes one or more fingertips with sudden movement onto body part of recipient, repetitive.
Pat (PT)	Subject rapidly and repeatedly contacts another individual with flattened palm surface of hand.
Touch (TO)	Any touch that does not fit into one of the above categories. Provide description.

Threat Gesture	**Description**
Arm Raise (AR)	Subject swings arm in a quick, upward motion towards another individual with palm facing down.
Wrist Shake (WS)	Subject shakes the hand vigorously and repeatedly with a flexible wrist towards another individual.

Other Gestures	**Description**
Arm Over/Hunchover (AO)	Subject sweeps one arm over the back of another individual but without hugging or extended contact.

Cage Bang (CB)	Subject uses hand to forcefully strike a substrate with the apparent intention of creating noise.
Throw (TH)	Subject uses hand to toss debris (dirt, feces, etc.) towards a recipient.
Clap (CL)	Subject contacts hands together forcefully to create noise.
Self Hit/Clasp Self (SH)	Subject crosses one or more hands across torso and slaps their own body repeatedly.
Rap Knuckles (RK)	Subject hits the knuckles of one or both hands against the ground or other substrate without creating a loud noise, repetitive.

3.3 Social Cognition Assessment Procedures

To assess joint attention abilities, I utilized procedures similar to those used previously for measuring an individual's response to behavioral requests (RBR) and initiation of behavioral requests (IBR) in chimpanzees (modified from Hopkins and Taglialatela, 2013). Social cognition testing took place during the day in the bonobos' typical home enclosures (approximately 08:00 – 18:00). All individuals were voluntarily separated from their social group into a separate enclosure prior to testing. All trials were video recorded and later scored for performance. Each subject completed 20 RBR and 20 IBR trials as described below. Order of RBR and IBR trials was randomized at the beginning of testing and the same random trial order was used for all subjects. At the end of every trial, regardless of performance, the experimenter handed the bonobo a few small pieces of food for participating in the trial.

3.3.1 Responding to a behavioral request (RBR)

Responding to a behavioral request (RBR). Each subject completed 10 trials in condition 1 (experimenter present) and 10 trials in condition 2 (experimenter absent). For all RBR trials, two pieces of equal-sized fabric (one colored blue and one colored red) were fixed to the inside of the subject's enclosure. In condition 1, the experimenter stationed the subject equidistant between the two fabric pieces, and sat directly in front of the subject, just outside the enclosure, roughly 1 meter away. Once the bonobo was stationed and the pieces of fabric were secure to the

mesh, the experimenter started the timer and began the trial by alternating gaze between the bonobo and the target piece of fabric (counterbalanced for side and color) while pointing to the piece of fabric and mimicking a bonobo vocalization. The bonobo had 60 seconds to respond to the request. A response was defined as a successful response if the bonobo removed the target piece of fabric and successfully pushed it through the mesh to give it to the experimenter. Each trial was scored as 0 if the subject never responded during the 60 seconds and scored as 1 if the subject responded at any point during the trial. For experimenter-present trials, videos were also coded for latency to respond to the behavioral request, time to complete the request, and whether or not the subject traded both pieces of fabric back (instead of just the target piece). Scores for each trial were summed such that each subject received a summary score ranging from 0 to 10 for condition 1. In condition 2 (experimenter absent), the experimenter stationed the bonobo between the two pieces of fabric and left the enclosure space. After 60 seconds, the experimenter returned, and the trial ended. Each trial in condition 2 was scored as 0 if the subject retrieved either piece of fabric and successfully pushed it through the enclosure mesh and scored as 1 if the subject did not retrieve either piece of fabric, or successfully push the piece of fabric through the mesh. Given that the social agent (experimenter) was absent, and no behavioral request had been made, it was considered non-social if the bonobo successfully pushed the piece of fabric outside of the enclosure in this condition. This condition was included to control for any non-social motivation the subject may have had to complete the task. Scores for each trial were summed resulting in a score ranging from 0 to 10.

3.3.2 Initiating a behavioral request (IBR)

Each subject completed 10 trials in condition 1 (experimenter present) and 10 trials in condition 2 (experimenter absent). For all IBR trials, two over-turned 2-gal buckets were

positioned roughly 1 meter from the outside of the subject's enclosure. In condition 1 (experimenter attentive), the experimenter stationed the subject equidistant between the buckets. The experimenter then placed a cup of sliced grapes – a high preference food item – on top of one of the buckets (counterbalanced for left and right side). Once the subject was stationed and the food item was placed on top of the bucket, the experimenter started the timer and directed her eye contact to the subject. During the 60-second trial, if the bonobo initiated a request, the attentive experimenter would re-direct her gaze to the food item and then back to the bonobo. A *request* was defined as the subject alternating their gaze between the experimenter and the food item while producing a manual gesture towards the food item. Each trial was scored as 0 if the subject never requested and scored as 1 if the subject made at least one request during the 60s trial. For all experimenter present trials, videos were coded for the latency to initiate the behavioral request and for the number of communicative signals directed toward the experimenter during the trial. Individual scores ranged from 0-10, with 10 being the highest achievable performance (subject initiated a request every trial). In condition 2 (experimenter absent), the experimenter stationed the bonobo equidistant between the two buckets, placed a cup of sliced grapes on top of one of the buckets (counterbalanced for side) and left the enclosure area. After 60 seconds, the experimenter returned, and the trial ended. Each trial in condition 2 (experimenter absent) was scored as 0 if the bonobo pointed to the food at any point during the trial and scored as 1 if the bonobo never pointed toward the food. Given that the social agent (experimenter) was absent and there is no one to initiate a behavioral request towards (i.e., there was no one to communicate with), it was considered non-social if the bonobo pointed towards the object outside of the enclosure in this condition. This condition was included as a control for

any non-social motivation the subject may have had to complete the task. Again, scores for each trial were summed resulting in a score ranging from 0 to 10.

3.4 Genetic Analyses

3.4.1 Gene Selection

The SFARI gene database was utilized (https://gene.sfari.org/) to identify genetic variants that are associated with the ASD phenotype in humans. All the genes from this list were entered into the NCBI protein blast program to verify if they are present in bonobos and if they have high percent identity and query cover. Next, the list of candidate genes was condensed further to only include genes that were ranked category 1 or 2 in association. Rankings of 1 or 2 are the highest levels of association on the SFARI gene database, meaning there is the most empirical evidence for an association between the gene and aspects of the ASD behavioral phenotype. Ultimately, three candidate genes were selected: arginine vasopressin receptor gene 1A (*AVPR1A*), oxytocin receptor (*OXTR*), forkhead box protein 2 (*FOXP2*). Next, all possible *AVPR1A*, *OXTR*, and *FOXP2* SNPs that have been associated with the behavioral phenotype of ASD were identified and put into the University of Santa Cruz (UCSC) genome browser gateway. The UCSC genome browser gateway includes the latest genomes for bonobos, chimpanzees, gorillas, and humans such as University of Washington May 2020 (Mhudiblu_PPA_v0/panPan3) for bonobos. Therefore, UCSC genome browser was used to visualize an alignment between the human and bonobo samples.

The DNA sequencer was then placed in NCBI primer blast to design primer pairs for DNA analysis. Each primer pair had to meet a set of requirements to qualify. This included: 1.) the PCR product had to be at least 250 base pairs and centered around the 250th base pair in the

template DNA sequence to ensure that the SNP location was included, 2.) the quality was correct, meaning Tm is around 60 degrees Celsius and GC% is around 50% = equal distribution of GC content and AT content, 3.) they had to pass a self-complimentary check - no hair-pin formations, not 3' prime complimentary, no self-annealing sites, and 4.) the primer pair needed to appear in the great apes' reference genome. The last requirement was verified using ApE – a sequence visualization tool that allows users to input their own sequence data and annotate it (https://jorgensen.biology.utah.edu/wayned/ape/) (Figure 3.4.1). The *AVPR1A*, *OXTR*, and *FOXP2* gene sequences were downloaded from the reference great ape genomes from the UCSC browser. Primer pairs that did not appear were excluded from the experiment. In the end, a total of 10 primer pairs were chosen for the bonobo genome. This included eight primer pairs for *OXTR*, one for *AVPR1A*, and one for *FOXP2*. Once checked, oligonucleotides were ordered for PCR from thermo fisher (Table 3.4.1).

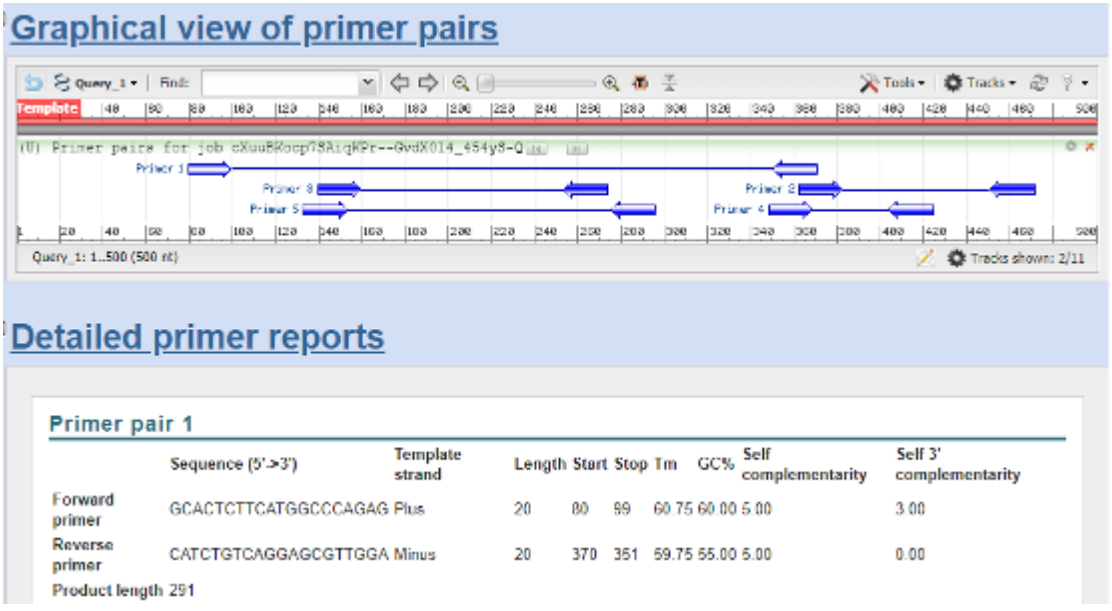

Figure 3.4.1 Visualization of the primer pair for rs53576

Table 3.2 Single Nucleotide Polymorphisms (SNPs) of Interest

Gene	SNP
AVPR1A	rs3803107
FOXP2	rs6980093
OXTR	rs2270463
OXTR	rs237877
OXTR	rs237878
OXTR	rs35062132
OXTR	rs2254295
OXTR	rs237894
OXTR	rs237895
OXTR	rs237900

3.4.2 DNA extraction, amplification, and sequencing

Biological samples were collected from 29 bonobos (6 whole blood samples and 23 buccal swabs). There were four objectives to the genetic analyses: 1.) DNA extraction from bonobo whole blood samples and buccal swabs, 2.) DNA amplification using PCR and gel electrophoresis, 3.) Gel purification to collect only desired DNA fragments, and 4.) Sequence collected DNA through GENEWHIZ. Specifically, whole blood samples were collected from 5 bonobos living at the Columbus Zoo and Aquarium and buccal swab samples were collected from 23 bonobos living at the Ape Cognition and Conservation Initiative and the Milwaukee County Zoo. For whole blood samples, 3 ml of blood was collected from each bonobo and stored in a freezer at -20 degrees Celsius. The blood lysate was prepared using the PureLink® Genomic DNA Mini Kit. The lysate started as a combination of 200 µl of frozen whole blood samples, 20 µl Proteinase K, and 20 µl RNase A in a 1.5 ml microcentrifuge tube. After that, the tube was vortexed briefly and incubated at room temperature for 2 minutes. Then, 200 µl PureLink® Genomic Lysis /Binding Buffer was added to the lysate, and the lysate was vortexed until

homogeneity. Once the lysate was homogenous, it was incubated at 55 degrees Celsius for 10 minutes in a hot bead bath to promote protein digestion. Then 200 µl 96-100% ethanol was added to the lysate, and vortexed by 5 seconds to yield a homogenous solution. The second step ensures that DNA binding conditions are still met while removing salt and protein contaminants. First, 96-100% ethanol was added to PureLink® Genomic Wash Buffer 1 and 2. Next, the lysate prepared with the genomic lysis/binding buffer and ethanol (~ 640 µl) was transferred to a PureLink® Spin Column in a collection tube. The column was then centrifuged at 10,000 *x g* for 1 minute at room temperature. Once the centrifuge cycle ended, the collection tube was discarded, and the spin column was placed in a new sterile collection tube.

For DNA washing, 500 µl of ethanol prepared wash buffer 1 was added to the spin column. Then the column was centrifuged at 10,000 *x g* at room temperature for 1 minute. The collection tube was discarded afterwards and replaced with a new sterile tube. The DNA was washed a second time with ethanol prepared wash buffer 2 and centrifuged at maximum speed, 15,000 *x g*, for 3 minutes at room temperature, and then the collection tube was discarded. To elute the DNA, the spin column was placed in a 1.5 ml microcentrifuge tube. Then 100 µl of PureLink® Genomic Elution Buffer was added to the column and incubated at room temperature for 1 minute before centrifugation at maximum speed. After centrifugation, the 1.5 ml microcentrifuge tube contained purified genomic DNA. These steps were performed for each whole blood bonobo sample.

For buccal swab samples, lysate was prepared differently. First, the Qiagen Omni Swab tips were ejected and placed in their own 2 ml microcentrifuge tube. Next, 600 µl of phosphate buffered saline (PBS) was added to the tube. Then, 20 µl of Proteinase K was added to a large 15 ml centrifuge tube, as well as 600 µl of swab lysate, which were mixed well via pipetting.

Afterwards, 600 µl of PureLink® Genomic Lysis/Binding Buffer was added to the lysate and mixed by vortexing. The lysate was incubated at 55 degrees Celsius in a hot bead bath for 15 minutes. The tube was centrifuged to collect any leftover lysate that may be on the cap, and then 200 µl of 96-100% ethanol was added to the lysate and vortexed for 5 seconds to get a homogenous solution. For binding DNA, washing DNA, and eluting DNA, all buccal swab samples were then processed using the same procedures as the whole blood samples.

At the end, the DNA concentrations extracted from the blood were checked with a nanodrop. First, the nanodrop was blanked out using distilled water twice, then each sample was tested. Next, standard/conventional PCR was used to amplify the target DNA sequence and PCR Master Mix (2X) from Thermo Scientific. A combination of 25 µl of PCR Master Mix, 1 µM of forward primer, 1 µM of reverse primer, and 1 µg of template DNA was then added to PCR tube kept on ice. Next, 22 µL of water was added to the mix making it a total of 50 µL. The mix ratio was repeated for each bonobo DNA sample and placed into the thermal cycler upon completion. The thermocycler conditions were set as follows:

1.) Initial denaturation - temperature: 94 degrees Celsius, time: 1 minute
2.) Denaturation – temperature: 94 degrees Celsius, time: 20 seconds
3.) Annealing – temperature: 55 degrees Celsius, time: 20 seconds
4.) Extension – temperature: 72 degrees Celsius, time: 20 seconds
5.) Final extension – 72 degrees Celsius, time: hold

The initial denaturation and final extension phases ran for one cycle each while the annealing and extension phases ran for a total of 35 cycles. Once the thermocycler completed the protocol, the PCR products were loaded onto 1.3% mini agarose gels for visualization. The visualized images served as a check to be sure that the PCR reaction was successful and

produced the target amplicon. The gel was made of 1.3 g of agarose, 100 ml of TAE buffer, and 2 µl of ethidium bromide. The TAE buffer was a combination of 20 ml TAE buffer, 980 ml of dH2O, and 2 µl of ethidium bromide. 250 ml of TAE buffer and 2.5 µl of ethidium bromide was added to the electrophoresis cell. For each well, the PCR product and purple loading dye was loaded with a ratio of 10:2 as suggested by the Quick-Load Purple 100 bp DNA ladder guide. The cells ran at 90 volts and 400 amps for 55 minutes. Afterwards the gels were placed in a Bio-Rad ChemiDoc XRS+ System.

The third objective, gel purification, was conducted using a Zymoclean™ Gel DNA Recovery kit. First, the gels were placed on a UV transilluminator. From there, each band was excised using a scalpel and transferred to a 1.5 ml microcentrifuge tubes. The microcentrifuge tubes' mass was 1.00 gram each. The total mass of the gel was calculated by taking the mass of the gel piece inside the microcentrifuge tube and subtracting the microcentrifuge tubes' mass. The mass number was then multiplied by 3 to figure out how many volumes (µl) of agarose dissolving buffer (ADB) to add to the microcentrifuge tube. Once ADB was added to the microcentrifuge tubes, they were incubated in a 55 degrees Celsius hot bead bath until the gel piece was completely dissolved and the solution was homogenous. Afterwards, the melted agarose solution was transferred to a Zymo-Spin™ Column in a collection tube and centrifuged for 60 seconds at 15,000 *x g*. The flow through was discarded from the collection tube so that it could be used again. For DNA washing, 24 ml of 96-100% ethanol was added to the 6 ml DNA wash buffer and 96 ml of 96-100% ethanol was added to the 24 ml DNA wash buffer. Next, 200 µl of DNA wash buffer was added to the column, and then the column was spun again for 30 seconds 15,000 *x g*. The flow through was discarded and the washing step was repeated. The final steps included placing the spin column into a 1.5 ml microcentrifuge tube, adding 10 µl of

DNA elution Buffer directly to the center of the spin column, and centrifuging for 60 seconds. The final mass of the purified DNA products was determined using both gel visualization (using 1 µl of DNA mixed with 2 µl dye and 3 µl water for clarity), and a nanodrop machine. The remaining extracted DNA from the gels were sent to GENEWHIZ for sequencing.

3.5 Data Analyses

3.5.1 Socio-communicative behavior and stereotypical/repetitive behavior

A social proximity score was calculated for each focal observation using the follow formula: ((3 * # touching data points) + (2 * # socially close data points) + (1 * # solitary data points) + (0 * # isolated data points)) / (11); 11 is the total number of proximity data points per focal observation. This resulted in a score that ranged from 0-3 for each focal observation, where higher scores indicate more proximity. Regarding communication, I summed the total number of vocalizations, gestures, facial expressions, and co-occurring signals produced during each focal follow (communicative production). I also summed the total number of stereotypical behaviors produced during each focal follow. Social proportion was calculated by summing the proportion of time spent engaged in grooming and engaged in play and dividing this by the total number of seconds in view during the focal follow (out of a possible 600 seconds). After raw values for all eight observations for all subjects were calculated, averages were created for each individual. Raw and averaged behavioral observations were included in separate analyses. Specifically, I divided social proximity score, communicative production, repetitive/abnormal behaviors, external gestures produced, and social proportion by 8 (number of observations per subject) to generate an average social proximity score, average communicative production value, average number of external gestures produced, average number of repetitive/abnormal behaviors produced, and average social proportion value for each of the 26 subjects.

3.5.2 Social cognition task performance

Total Scores for both RBR and IBR were calculated using the following formula: (summed score in the attentive condition + summed score in the absent condition)/2 = individual score ranging from 0 – 10. Average scores for latency to respond, latency to initiate, and time to complete the request were calculated for each individual, as well as an average number of communicative signals produced during the experimental present IBR trials.

3.5.3 Bonobo SNP identification

To determine whether or not SNPs in the human genome that are associated with ASD are also SNPs in bonobos, I utilized the UCSC genome browser (http://genome.ucsc.edu) to compare each individual bonobo's sample to the human samples reference genome [Dec. 2013 (GRCh38/hg38)]. Specifically, individual Sanger Sequencing results for each subject were imported into the UCSC browser and then I aligned individual sequence files to the human reference genome [Dec. 2013 (GRCh38/hg38)] using the BLAT tool (Kent, 2002). The location of each SNP was then identified on the human reference genome, and the corresponding nucleotide in each bonobo subject was recorded.

3.5.4 The Relationship Between Social Behavior and Communication

To determine whether a putative relationship between social behavior and communicative production exists at the individual-level, I conducted a repeated measures correlation (rmcorr) on the **raw** focal observation data from all 26 subjects (8 observations per subject * 26 subjects = 208 focal observations). Specifically, I utilized a rmcorr analysis in RStudio v1.4.1717 with social proximity score, communicative production, number of repetitive/abnormal behaviors, number of external gestures produced (i.e., gestures directed toward the experimenter and not a conspecific), and social proportion (i.e., proportion of time spent engaging in play or grooming

with conspecifics) as the dependent variables. This analysis was conducting separately for females and for males given the well documented sex differences in socio-communicative behavior for bonobos.

To determine whether or not facility and sex could be accounting for individual differences in socio-communicative behavior, I utilized a Multivariate Analysis of Variance (MANOVA) on the **averaged** observation data per subject. Specifically, I included sex and facility as the fixed factors, and average social proximity score, average communicative production, average number of repetitive/abnormal behaviors produced, average number of external gestures produced, and average social proportion as the dependent variables.

3.5.5 Socio-Communicative Behavior and Socio-Cognitive Functioning

To determine whether there is a connection between observable socio-communicative behavior and joint attention abilities, I conducted Pearson correlations on the averaged focal observation data and joint attention task performance data for the 7 bonobos living at Ape Initiative. Specifically, I utilized a partial correlation in SPSS v27 with average social proximity score, total communicative production, repetitive/abnormal behaviors, and social proportion as the behavioral dependent variables and average RBR score, IBR score, latency to initiate, latency to respond, and time to complete the request as the cognitive dependent variables, while controlling for sex. Bonferroni's correction for multiple comparisons indicated an a priori significance threshold = (0.05/9) = 0.0.

3.5.6 The Genetic Basis of Socio-Communicative Behavior and Social Cognition

To determine whether or not specific SNPs in bonobos predict individual-level socio-communicative behavior and joint attention abilities, I planned to utilize a linear regression analysis. Specifically, I planned to conduct a linear regression in SPSS v27 with average social

proximity score, communicative signals produced, repetitive/abnormal behaviors, and social proportion as the dependent variables and SNP allele as the predictor variable.

4 RESULTS

4.1 The Relationship Between Social Behavior and Communication

A repeated measures correlation (rmcorr) on the 112 female behavioral observations and 96 male behavioral observations revealed a significant negative correlation between social proportion (time spent engaged in grooming or play) and repetitive/abnormal behaviors produced (r (97) = -0.202, p = 0.045), between social proportion and external gestures produced (r (97) = -0.264, p = 0.008), and between proximity score (how close the individual was to conspecific social partners) and external gestures produced (r (97) = -0.224, p = 0.025) in female bonobos (Figures 4.1.1-4.1.3). In addition, rmcorr analyses revealed a significant negative association between communicative signals produced and grooming proportion (time spent engaged in grooming, r (97) = -0.217, p = 0.031), as well as significant positive association between the number of gestures produced toward conspecifics and play proportion (time spent engaged in play, r (97) = 0.396, p < 0.001) in female bonobos. For male bonobos, rmcorr analyses also indicated a significant positive association between the number of gestures produced towards

conspecifics and play proportion (r (83) = 0.335, p = 0.002), as well as a trend for a positive association proximity score and communicative production (r (83) = 0.187, p = 0.087)

In addition, MANOVA results reveled a significant main effect for facility, F (10, 32) = 2.916, p = 0.10; Wilk's Λ = 0.274, partial $\eta 2$ = 0.477 (Figure 4.1.7). Specifically, facility had a significant effect on social proximity score, F (2, 20) = 3.775, p =0.041, partial $\eta 2$ = 0.274; external gestures produced, F (2, 20) = 9.862, p =0.001, partial $\eta 2$ = 0.497; and total communicative production, F (2, 20) = 3.925, p = 0.036, partial $\eta 2$ = 0.282. A Tukey HSD post-hoc test indicated that the average social proximity scores differed significantly between Ape Initiative (AI) and Columbus Zoo and Gardens (CZG; p = 0.019) and between Milwaukee County Zoo (MCZ) and CZG (p = 0.032), but not between AI and MCZ (p = 0.818). Specifically, average social proximity score was higher for CGZ than either of the other two facilities. The average social proximity scores for AI = 1.61, CGZ = 2.22, and MCZ = 1.72, with a higher score indicating closer proximity. In addition, these analyses indicated that average communicative production differed between AI and CZG (p = 0.025), but not between AI and MCZ (p = 0.062) MCZ and CZG (p = 0.990). The average number of communicative signals produced (communicative production) for AI = 8.61, CGZ = 3.57, and MCZ 3.32. Furthermore, post-hoc tests indicated that the average number of external gestures produced toward the experimenter (external gestures) differed between AI and MCZ (AI > CGZ; p < 0.001), and between AI and CZG (AI > CGZ; p = 0.001), but not between CZG and MCZ (0.738). The average number of external gestures produced toward the experimenter for AI = 1.18, CGZ = 0.00, and MCZ = 0.18.

Additionally, a MANOVA revealed a significant main effect for sex, F (5, 16) = 4.206, p = 0.012; Wilk's Λ = 0.432, partial $\eta 2$ = 0.568 (Figure 4.1.8). Specifically, significant differences

between males and females were observed for social proximity score (F (1, 20) = 17.110, p < 0.001; partial η2 = 0.461). The average social proximity score for females = 2.11 and males = 1.50. However, the MANOVA also revealed a significant interaction between facility and sex on external gestures produced, F (2, 20) = 4.166, p = 0.031, partial η2 = 0.294 (Figure 4.1.9). Specifically, the average number of external gestures produced for AI males = 1.72, AI females = 0.46, CGZ males = 0.00 CGZ females = 0.00, MCZ males = 0.15, MCZ females = 0.20. AI males produced significantly more external gestures than all other males and females living at CGZ (p < 0.001) and MCZ (p = 0.001).

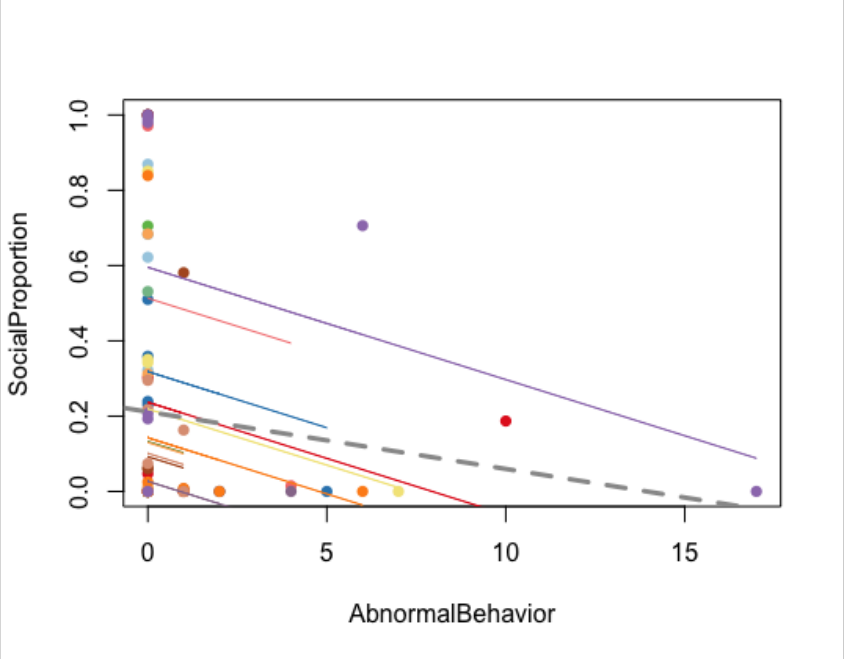

Figure 4.1.1 Negative Repeated Measures Correlation Between Repetitive/Abnormal Behaviors and Social Proportion
*Each color represents a female bonobo

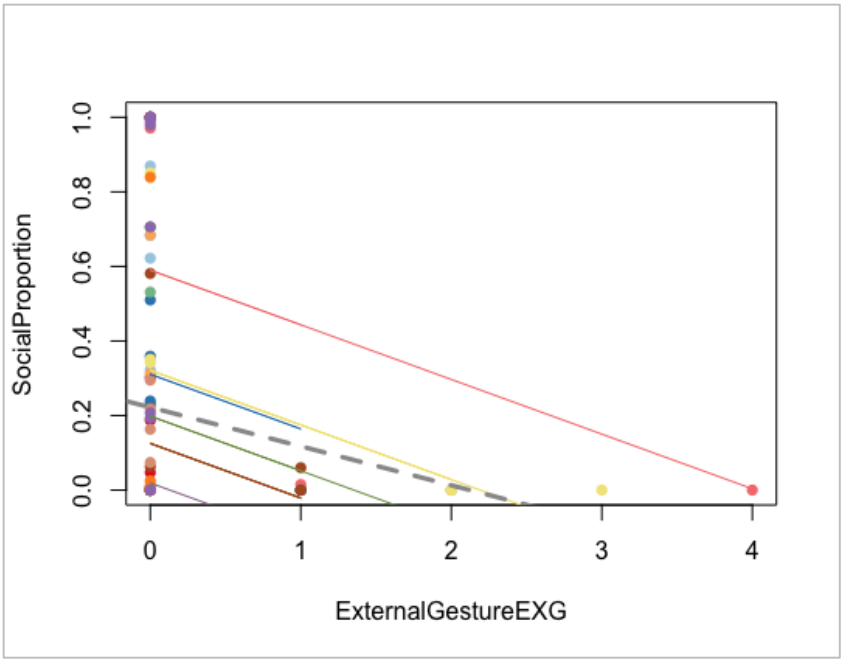

Figure 4.2.2 Negative Repeated Measures Correlation Between External Gestures Produced and Social Proportion
*Each color represents a female bonobo

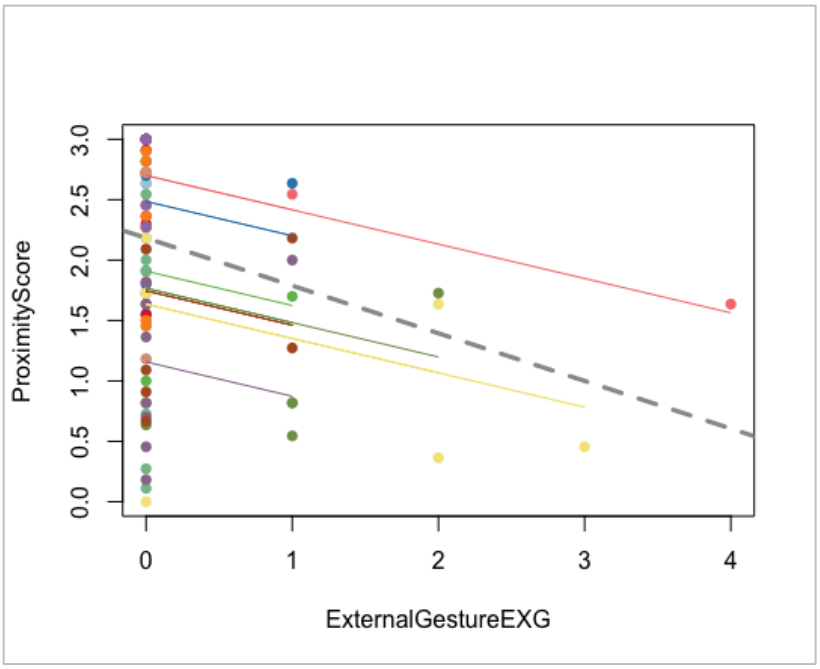

Figure 4.3.3 Negative Repeated Measures Correlation Between External Gestures Produced and Proximity Score
*Each color represents a female bonobo

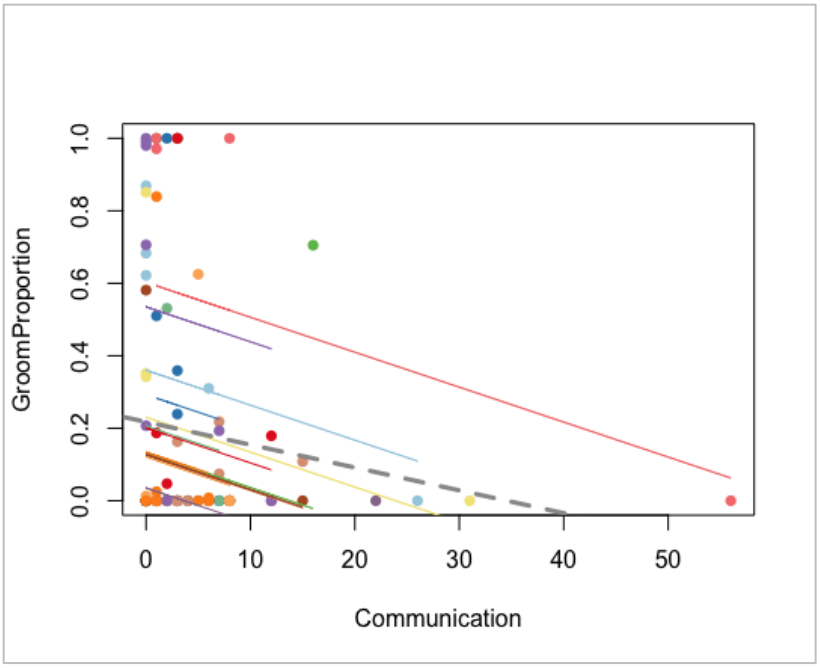

Figure 4.4.4 Negative Repeated Measures Correlation Between Communicative Production and Grooming Proportion
*Each color represents a female bonobo

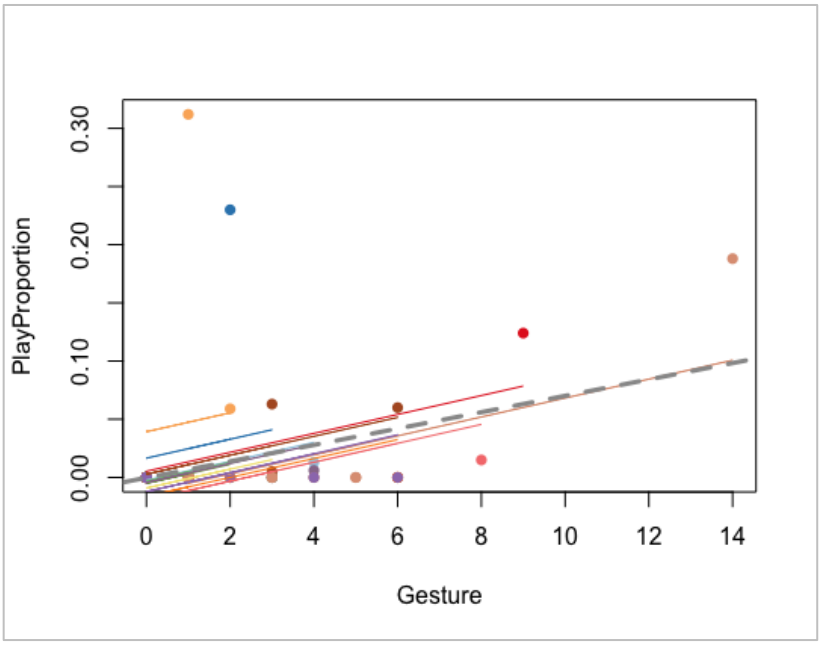

Figure 4.5.5 Positive Repeated Measures Correlation Between Gestures Produced and Play Proportion
*Each color represents a female bonobo

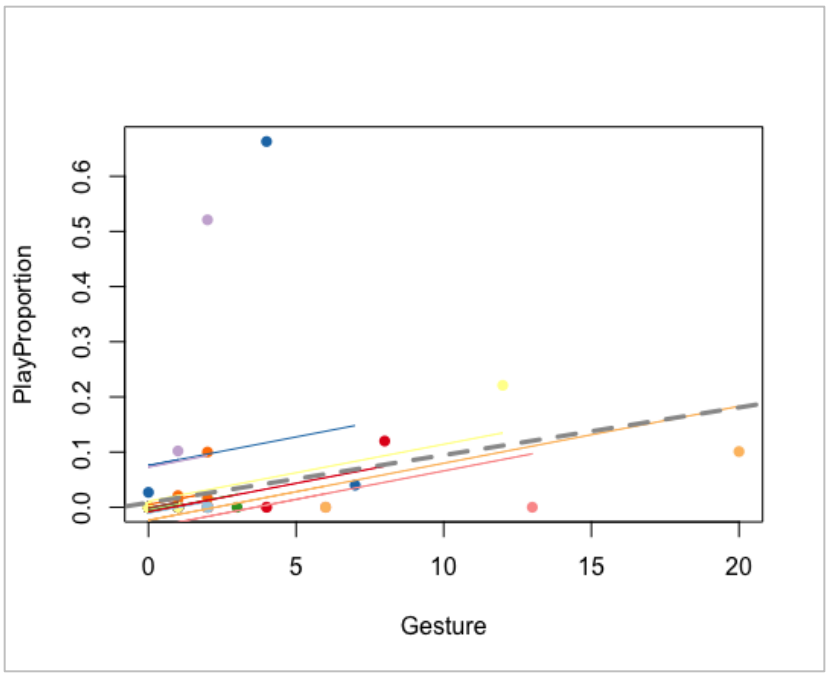

Figure 4.6.6 Positive Repeated Measures Correlation Between Gestures Produced and Play Proportion
*Each color represents a male bonobo

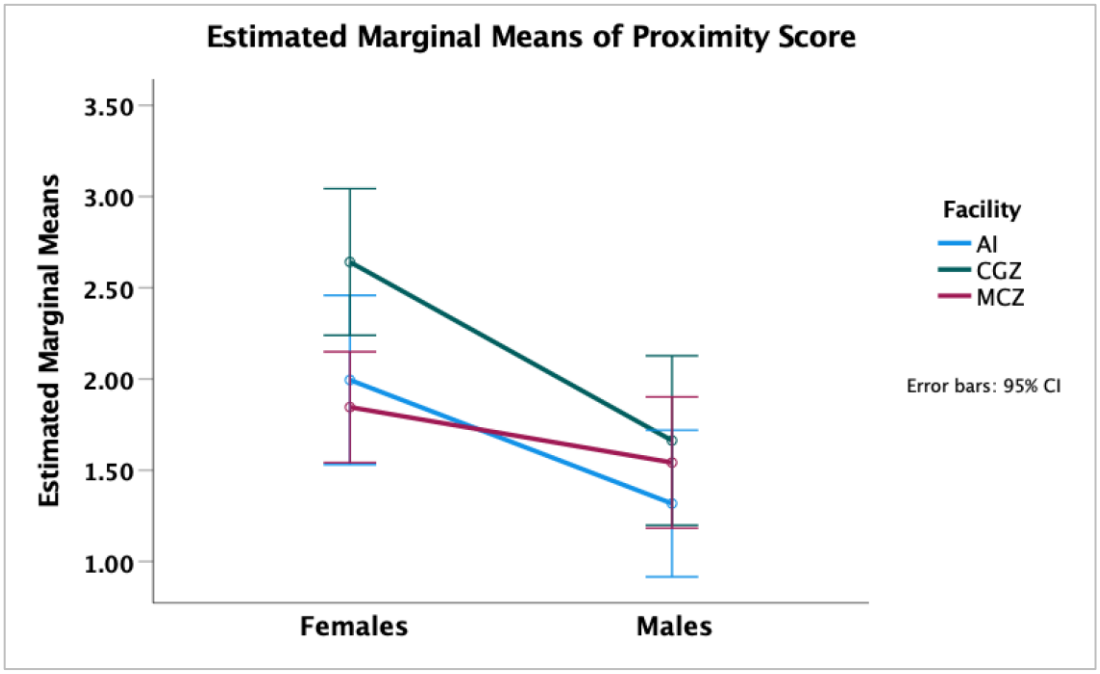

Figure 4.7.7 Estimated Marginal Means of Social Proximity Score

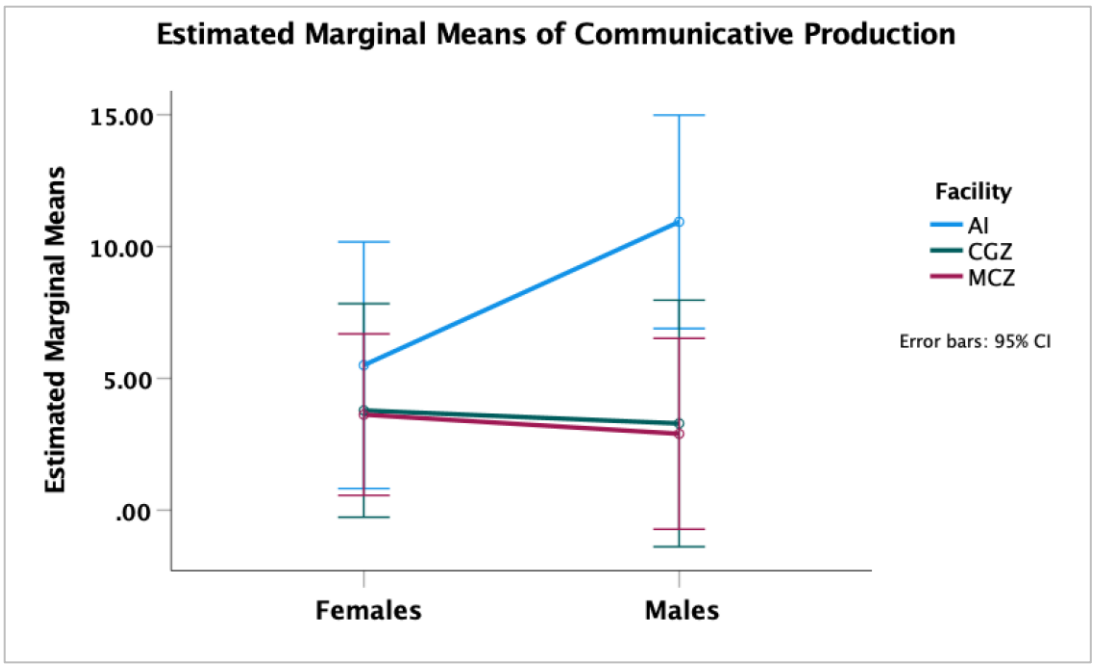

Figure 4.8.8 Estimated Marginal Means of Communicative Production

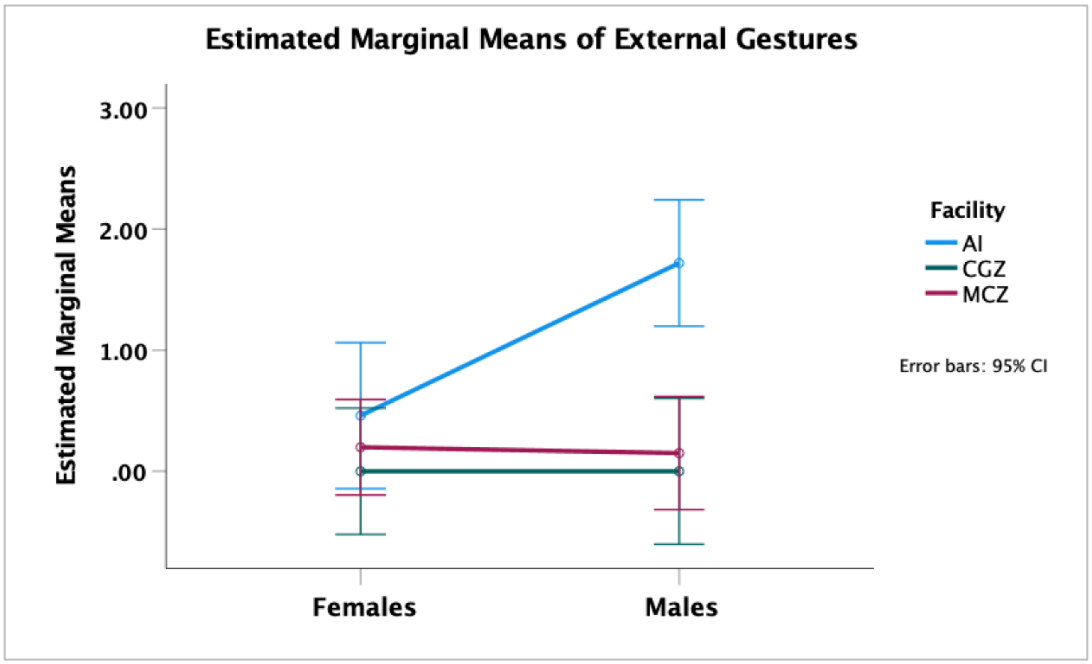

Figure 4.9.9 Interaction between Sex and Facility for External Gestures Produced

Table 4.1 Mean (Standard Error) for All Dependent Variables for Each Individual

Subject	Facility	Sex	Age	Social Proximity	Communicative Production	External Gestures	Abnormal Behaviors	Social Proportion
7	AI	Female	10	1.84 (0.28)	4.63 (2.03)	0.25 (0.16)	0	0.09 (0.09)
11	AI	Female	13	2.52 (0.20)	11.00 (6.52)	0.63 (0.50)	0.50 (0.50)	0.50 (0.19)
8	AI	Female	23	1.63 (0.37)	0.88 (0.40)	0.50 (0.27)	0.25 (0.16)	0.13 (0.13)
13	AI	Male	10	1.06 (0.36)	8.50 (1.72)	0.63 (0.26)	0.38 (0.26)	0.09 (0.07)
10	AI	Male	20	1.35 (0.31)	11.38 (4.17)	1.25 (0.49)	0.13 (0.13)	0.14 (0.12)
12	AI	Male	22	1.30 (0.38)	1.88 (1.74)	1.63 (0.96)	0	0.04 (0.03)
9	AI	Male	40	1.57 (0.31)	22.00 (8.15)	3.37 (1.57)	0	0.11 (0.11)
17	CZG	Female	11	2.68 (0.18)	4.25 (1.21)	0	0.13 (0.13)	0.13 (0.09)
18	CZG	Female	25	2.38 (0.22)	2.63 (0.91)	0	1.13 (0.74)	0.11 (0.10)
21	CZG	Female	24	2.84 (0.11)	2.63 (1.59)	0	2.88 (2.15)	0.51 (0.16)
20	CZG	Female	35	2.67 (0.22)	5.63 (1.48)	0	0.25 (0.16)	0.09 (0.04)
15	CZG	Male	8	2.10 (0.22)	5.50 (3.12)	0	0.75 (0.62)	0.27 (0.13)
19	CZG	Male	23	1.51 (0.18)	2.63 (2.49)	0	0	0.03 (0.03)
16	CZG	Male	32	1.38 (0.32)	1.75 (0.70)	0	2.00 (1.32)	0.09 (0.09)
25	MCZ	Female	10	1.67 (0.28)	5.63 (2.04)	0.25 (0.16)	0.13	0.09 (0.07)
1	MCZ	Female	15	2.15 (0.21)	5.13 (3.15)	0	0	0.31 (0.13)
3	MCZ	Female	15	2.45 (0.21)	3.00 (0.63)	0.13 (0.13)	0.88 (0.64)	0.29 (0.12)
14	MCZ	Female	17	2.47(0.22)	2.50 (1.40)	0	1.50 (1.23)	0.19 (0.12)
22	MCZ	Female	21	1.12 (0.24)	3.75 (2.81)	0.13 (0.13)	0.88 (0.52)	0
5	MCZ	Female	23	1.67 (0.29)	1.50 (0.40)	0	0	0.19 (0.16)
24	MCZ	Female	53	1.39 (0.36)	3.88 (3.88)	0.88 (0.44)	0.87	0.19 (0.11)
2	MCZ	Male	9	1.47 (0.27)	3.00 (1.52)	0	0.50 (0.50)	0.09 (0.08)
6	MCZ	Male	22	1.59 (0.32)	3.37 (1.12)	0.25 (0.16)	1.50 (0.71)	0
4	MCZ	Male	25	2.01 (0.29)	1.13 (0.40)	0	0	0.38 (0.16)
23	MCZ	Male	30	1.42 (0.43)	1.50 (1.23)	0	0	0.25 (0.16)
0	MCZ	Male	31	1.22 (0.28)	5.50 (4.11)	0.50 (0.27)	0.38 (0.26)	0.14 (0.12)

4.2 Socio-Communicative Behavior and Socio-Cognitive Functioning

A partial Pearson correlation analyses on the average behavioral observation data and the average joint attention data for the 7 bonobos living at Ape Initiative (after accounting for sex) revealed a significant negative correlation between average communicative signals produced and average latency to complete the behavioral request (RBR), $r(4) = -0.945$, $p = 0.005$ (Figure 4.2.1), as well as a significant positive association between the average number of external signals produced during observations and the average number of signals produced during the experimenter present initiating joint attention trials, $r(4) = 0.951$, $p = 0.004$ (Figure 4.4.2).

Table 4.2.2 Average Scores for Initiating and Responding to a Behavioral Request – Overall and in the Experimenter Present and Experimenter Absent Conditions

Subject	Sex	Age	IBR Score	RBR Score	Present Score	Absent Score
Clara	Female	10	8	7	8.5	6.5
Mali	Female	13	9	9	9.5	8.5
Elikya	Female	23	9	9.5	10	8.5
Teco	Male	10	9.5	7	9.5	7
Maisha	Male	20	9.5	10	9.5	10
Nyota	Male	22	9.5	7.5	10	7
Kanzi	Male	40	5	5.5	10	0.5

Table 4.2.2 Mean (SE) for Behavioral Variables (Blue) and Cognitive Variables (Peach)

Subject	Sex	Age	Social Proximity	Communicative Production	External Gestures	Number of Signals (IBR-P)	Latency to Initiate	Latency to Respond	Completion Time (RBR-P)
Clara	Female	10	1.84 (0.28)	4.63 (2.03)	0.25 (0.16)	1.80 (0.55)	21.48 (8.46)	1.56 (0.41)	7.68 (0.47)
Mali	Female	13	2.52 (0.20)	11.00 (6.52)	0.63 (0.50)	2.90 (0.72)	16.42 (5.87)	2.31 (0.71)	6.34 (1.14)
Elikya	Female	23	1.63 (0.37)	0.88 (0.40)	0.50 (0.27)	4.90 (0.90)	4.09 (0.69)	2.19 (0.90)	10.59 (2.14)
Teco	Male	10	1.06 (0.36)	8.50 (1.72)	0.63 (0.26)	3.50 (0.81)	13.34 (5.75)	1.23 (0.17)	9.12 (1.36)
Maisha	Male	20	1.35 (0.31)	11.38 (4.17)	1.25 (0.49)	3.70 (0.65)	18.97 (5.32)	1.34 (0.11)	9.24 (2.02)
Nyota	Male	22	1.30 (0.38)	1.88 (1.74)	1.63 (0.96)	6.70 (0.88)	6.16 (1.42)	1.48 (0.134)	10.57 (2.07)
Kanzi	Male	40	1.57 (0.31)	22.00 (8.15)	3.37 (1.57)	13.00 (0.86)	2.86 (0.69)	1.67 (0.30)	5.03 (0.80)

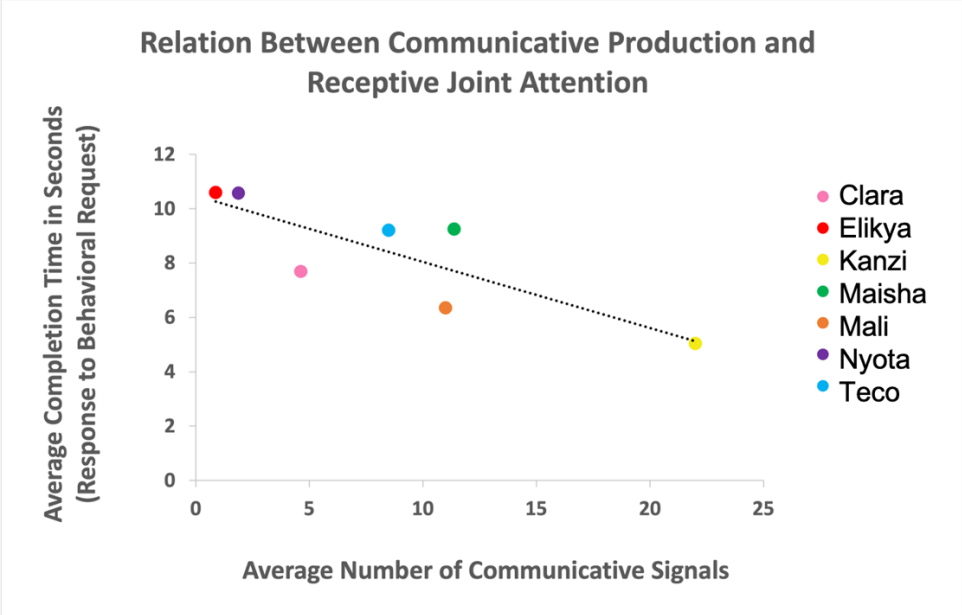

Figure 4.2.1 Correlation Between Average Communicative Production During Behavioral Observations and Average Completion Time for Receptive Joint Attention – Experimenter Present Trials

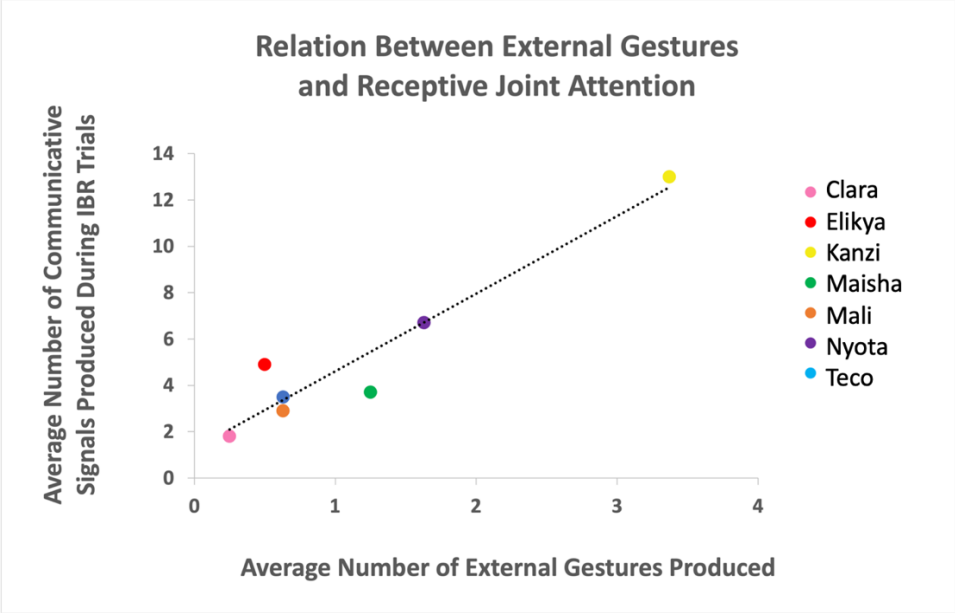

Figure 4.2.2 Correlation Between Average External Gestures Produced During Behavioral Observations and Average Communicative Signals Produced During Initiating Joint Attention – Experimenter Present Trials

4.3 The Genetic Basis of Socio-Communicative Behavior and Social Cognition

For all ten of the *AVPR1A*, *FOXP2*, and *OXTR* SNPs associated with ASD in humans, the bonobos did not differ at the individual-level (Figures 4.3.1 - 4.3.10). These figures include the visualizations between individual bonobo samples and the human reference genome. As indicated in Figure 4.3.2, even for human SNPs where the humans and bonobos differ (*FOXP2*; human allele = G and bonobo allele = A), all of the bonobo samples contained the same nucleotide. A linear regression analysis could not be conducted, given there was no variation in the nucleotide present at each corresponding human SNP. For 6 out of the 10 target variants, all of the bonobos exhibited the dominant human allele, whereas for the other 4 variants (*FOXP2* rs6980093, *OXTR* rs237877, *OXTR* rs237878, *OXTR* rs237895), they all exhibited the alternate human allele (risk allele – the allele associated with ASD). See Table 4.3.1 for a full list of allele locations, frequencies, and functions, and Table 4.3.2 for the coefficients of relatedness between subjects.

Although the nucleotides at each of the 10 human SNP sites did not differ between individual bonobos, results from visualization with the UCSC Genome Browser indicate one adult, female bonobo (Lady from CGZ) does differ from all of the other bonobos in regard to the *OXTR* rs201778590 – a known SNP in humans (Figure 4.3.11). A list of all of the *AVPR1A*, *FOXP2*, and *OXTR* human SNPs and the human and bonobo alleles are listed in Table 4.3.

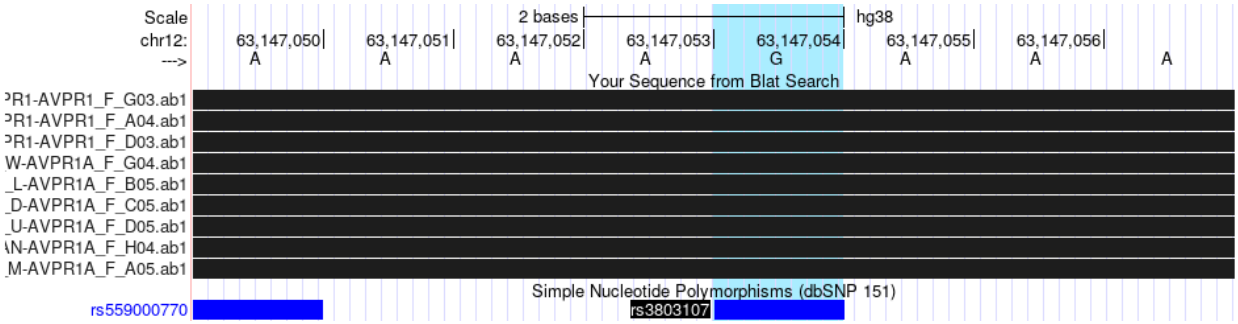

Figure 4.3.10 Visualization of Individual Bonobo Samples to Human Reference Genome for AVPR1A rs3803107 (n=9)

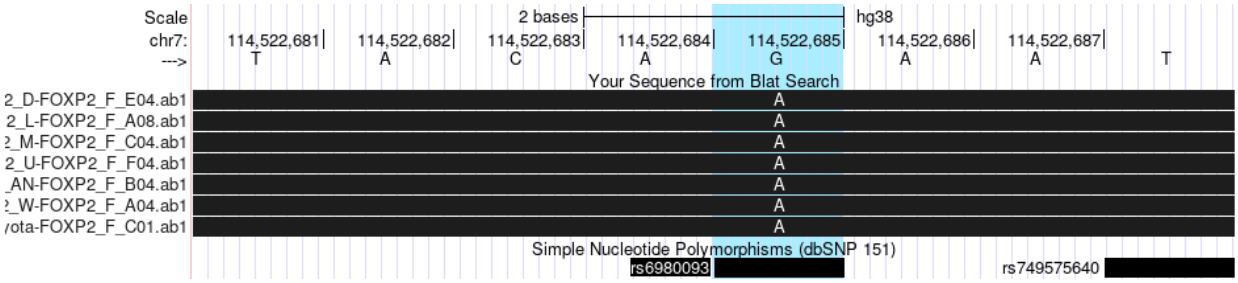

Figure 4.3.2 Visualization of Individual Bonobo Samples to Human Reference Genome for FOXP2 rs6980093 (n=7)

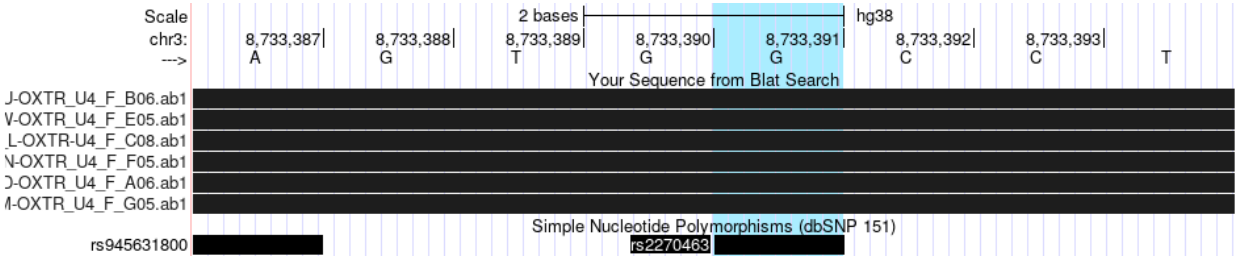

Figure 4.3.3 Visualization of Individual Bonobo Samples to Human Reference Genome for OXTR rs2270463 (n=6)

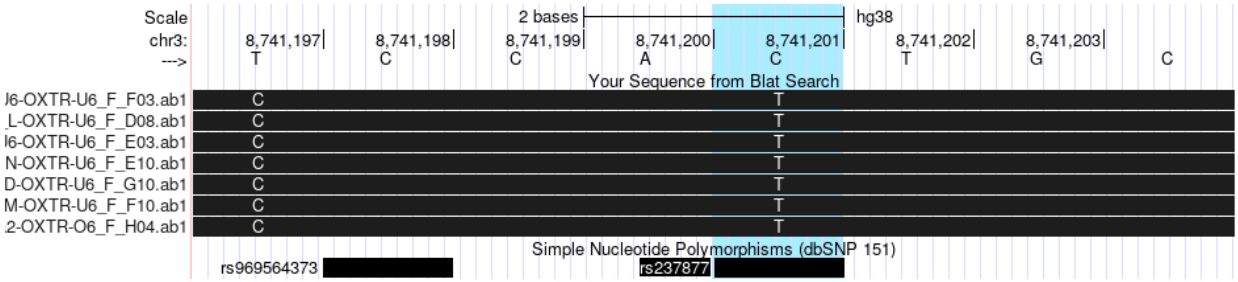

Figure 4.3.4 Visualization of Individual Bonobo Samples to Human Reference Genome for OXTR rs237877 (n=5)

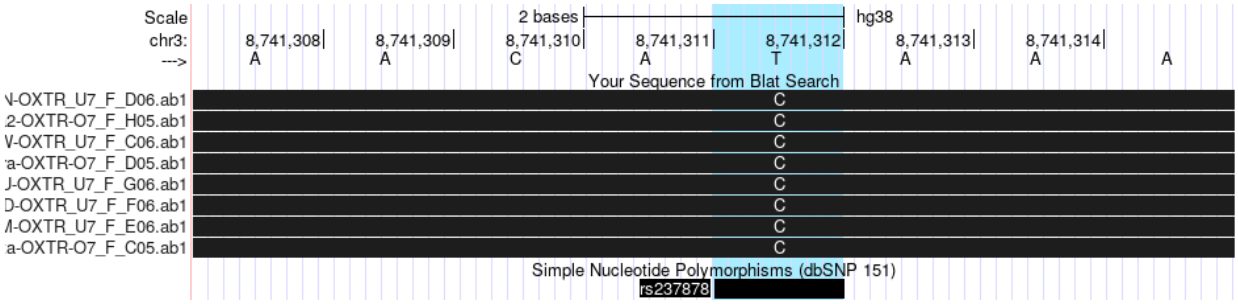

Figure 4.3.5 Visualization of Individual Bonobo Samples to Human Reference Genome for OXTR rs237878 (n=8)

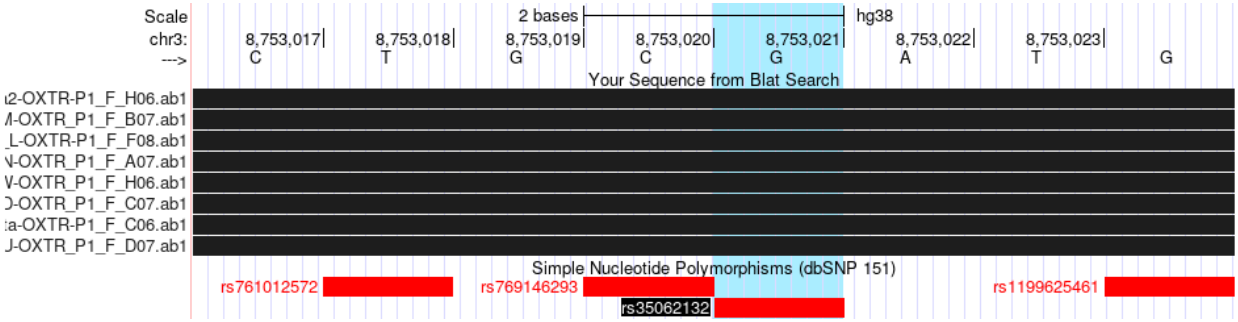

Figure 4.3.6 Visualization of Individual Bonobo Samples to Human Reference Genome for OXTR rs35062132 (n=8)

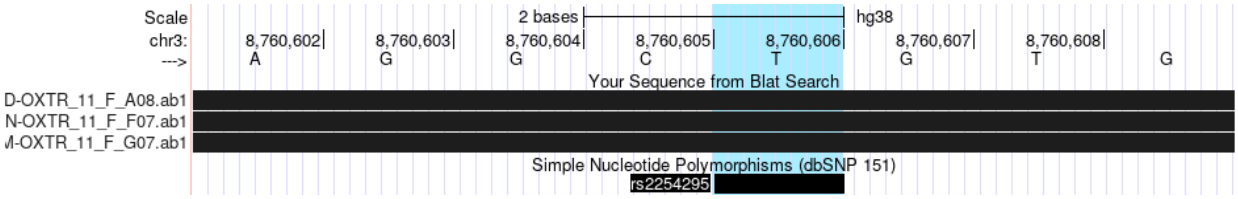

Figure 4.3.7 Visualization of Individual Bonobo Samples to Human Reference Genome for OXTR rs2254295 (n=3)

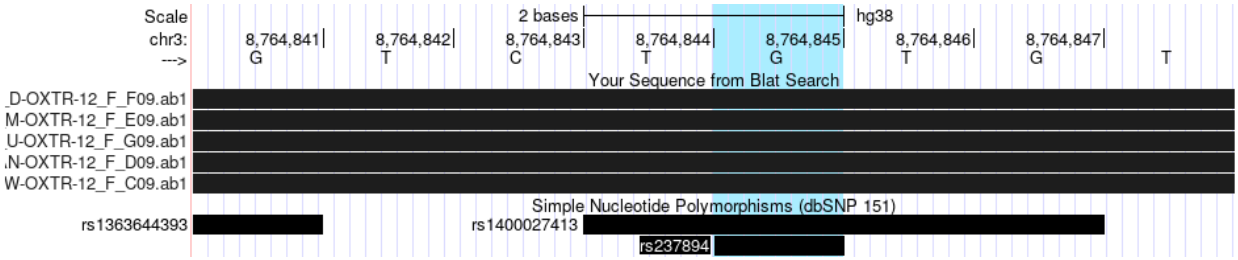

Figure 4.3.8 Visualization of Individual Bonobo Samples to Human Reference Genome for OXTR rs237894 (n=5)

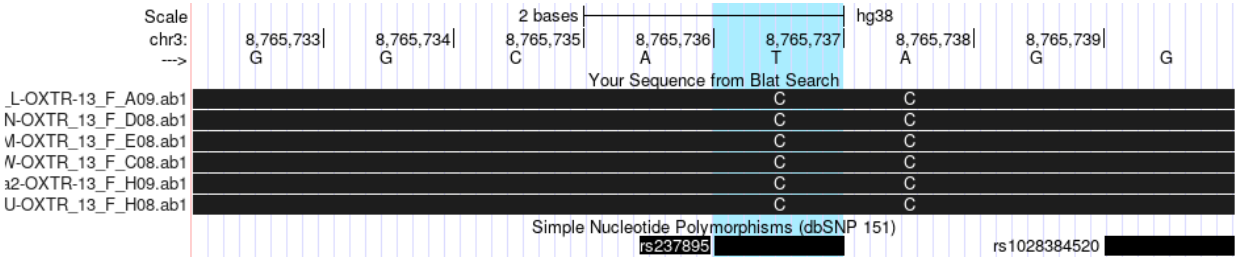

Figure 4.3.9 Visualization of Individual Bonobo Samples to Human Reference Genome for OXTR rs237895 (n=6)

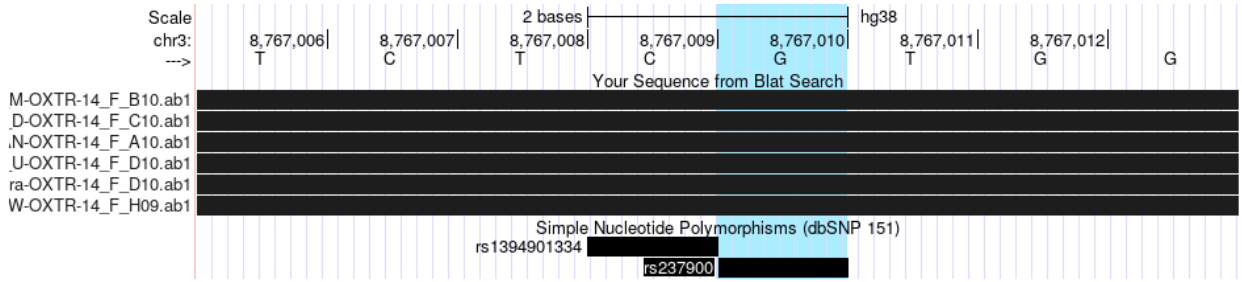

Figure 4.3.10 Visualization of Individual Bonobo Samples to Human Reference Genome for OXTR rs237900 (n=6)

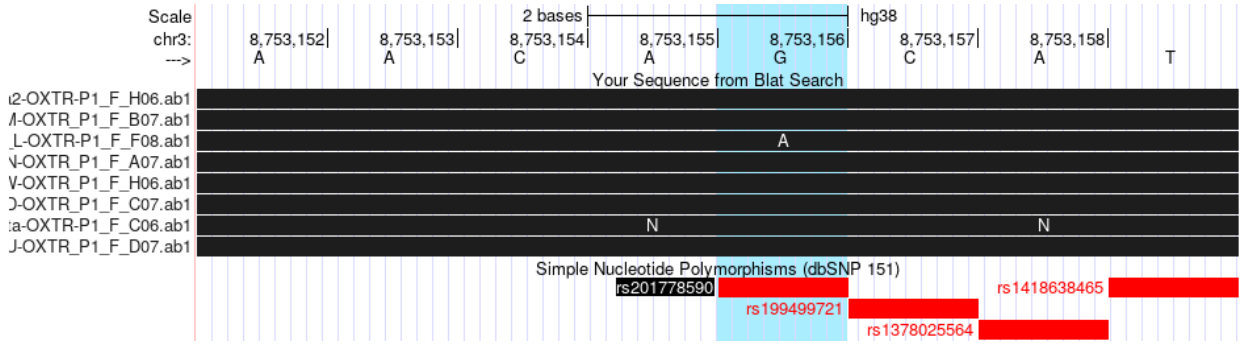

Figure 4.3.11 Visualization for OXTR rs201778590 (Lady Allele = A, Common Bonobo Allele = G, Common Human Allele = G, Rare Human Allele = A)

Table 4.3.1 Position, Frequency, and Function of SNPs of Interest that are Associated with ASD in Humans

Gene	SNP	Position (HRG)	Alleles (Frequency)	Function
AVPR1A	rs3803107	chr12:63147054	G (.83) / A (.17)	3' UTR Variant
FOXP2	rs6980093	chr7:114522685	G (.41) / A (.59)	Intron Variant
OXTR	rs2270463	chr3:8733391	G (.77) / T (.23)	Intron Variant
OXTR	rs237877	chr3:8741201	C (.69) / T (.31)	Intron Variant
OXTR	rs237878	chr3:8741312	T (.78) / C (.22) / A (.00)	Intron Variant
OXTR	rs35062132	chr3:8753021	G (1.00) / A (.00) / C (.00)	Missense Variant
OXTR	rs2254295	chr3:8760606	T (.87) / C (.13)	Intron Variant
OXTR	rs237894	chr3:8764845	G (.76) / C (.24)	Intron Variant
OXTR	rs237895	chr3:8765737	T (.37) / C (.63)	Intron Variant
OXTR	rs237900	chr3:8767010	G (.64) / A (.36)	Intron Variant
OXTR	rs201778590	chr3:8753156	G (1.00) / A (.00) / C (.00)	Missense Variant

Allele position, frequency, and function based on https://www.ncbi.nlm.nih.gov/snp/
HRG – Human Reference Genome [Dec. 2013 (GRCh38/hg38)]
Green – Bonobos have the alternate human allele
Red – Human SNP that is also a potential SNP in bonobo OXTR

Table 4.3.2 Coefficients of Relatedness Between Females (Vertical) and Males (Horizontal) Included in the Genetic Analyses

	Donnie	Max	Noki	Nyota	Wilber
Ana Neema	0	0	0	0.063	0.25
Clara	0	0	0.031	0	0
Elikya	0	0	0	0.188	0.063
Lady	0	0.25	0.125	0	0
Nora	0.063	0.125	0.063	0	0.031
Unga	0	0	0	0	0
Zomi	0	0	0.063	0	0

5 DISCUSSION

Typical socio-communicative development involves selective attention to social stimuli, intrinsic motivation to engage with social partners, and the use of communicative signals during social interactions. Although it is clear that genetic, behavioral, and cognitive factors all contribute to socio-communicative development in humans, it remains a significant challenge to disentangle the contribution of each to the emergence of socio-communicative abilities. Interestingly, a burgeoning body of literature highlights the unique value of studying these mechanisms in *nonhuman* animals, especially those most closely related to humans (Donaldson et al., 2008; Hammock & Young, 2006; Staes et al., 2018).

To this end, I investigated whether or not the putative relation between social behavior, communication, abnormal/repetitive behavior, and social cognition exists in one of the species most closely related to humans –bonobos. The present findings indicate there is a negative association between social behavior and repetitive/abnormal behavior for female bonobos. Specifically, the less time females spent engaged in grooming or play, the more repetitive/abnormal behaviors they produced. This finding suggests that much like in the case of humans diagnosed with ASD, level of social engagement could be linked directly to rates of abnormal/repetitive behaviors. It is interesting that this is the first evidence of a relation between individual-level social behavior and repetitive/abnormal behavior in bonobos. The present finding is complimentary to previous evidence that female bonobos' self-directed hair plucking is associated with urinary cortisol (physiological indicator of stress) and is consistent with literature on the behavioral phenotype of ASD and other neurodevelopmental disorders (Brand, et al., 2016; Jiujias, Kelley & Hall, 2017; American Psychological Association, 2013). For example, Richler and colleagues found that socio-communicative impairments were associated

with more severe repetitive behaviors in children diagnosed with ASD (Richler, Huerta, Bishop & Lord, 2010). Collectively, these results highlight the complex relationships between aspects of social behavior and communication at the individual level. Furthermore, they suggest that bonobos may be the ideal model for testing hypotheses about the complex behavioral phenotypes associated with neurodevelopmental disorders, such as ASD.

In addition to a negative relation between social behavior and repetitive/abnormal behavior, there is also a negative relation between external gestures and social behavior in female bonobos. In other words, the more individuals tried to engage with the human experimenter, the less time they spent engaging in grooming and play with, or in close proximity to, their conspecific social partners. This finding may reflect a potential tradeoff between intra and inter-specific social engagement. For example, even for the most social and communicative nonhuman great ape species, there is only so much time to spend interacting with social partners. Therefore, individuals are likely selective about which social partners they choose to spend the most time engaging with. For some of the bonobos, like those that were raised by humans, there may be a preference for inter-specific over intra-specific communication and social engagement.

When considering specific types of communicative signals, researchers have hypothesized that bonobos rely more on auditory communication when they are out of close visual proximity from one another (Furuichi, 2009). In contrast, bonobos are thought to use visual communication (e.g., gestures) more often when they are in close visual proximity to one another – like when they are playing. Indeed, in the current study both male and female bonobos exhibited a positive association between the proportion of time spent playing with conspecifics and the number of gestures produced. Specifically, bonobos that spent more time engaged in play were producing significantly more gestures than individuals that spent less time playing. These

findings provide additional evidence that bonobos modify their communication based on aspects of their social environment (Clay & Zuberbühler, 2012).

An additional relation between social behavior and communication was found in female bonobos. Specifically, female bonobos that spent less time grooming with conspecifics produced more communicative signals. This finding is consistent with theories proposing that complex communication replaced manual grooming, within the hominin lineage, as a way to maintain strong bonds in large social groups (Leavens, et al., 2014; Dunbar, 1996). Previous work has demonstrated that grooming not only provides the receiver with physiological benefits (e.g., the removal of dead skin and parasites), but it also provides the groomer with social benefits, such as support from other individuals during agonistic interactions (Stevens et al., 2005; Watts, 2000). In social primates, grooming rates increase as group size increases; however there appears to be a limit to the number of social partners that can be attended to with manual grooming (Grueter et al., 2013; Lehmann et al., 2007; Dunbar, 1996). Specifically, when group size exceeds a critical number, manually grooming each socially partner becomes unrealistic given the amount of time necessary for survival behaviors, such as foraging, (Dunbar, 1996; Lehmann et al., 2007; Leavens et al., 2014). Therefore, it is hypothesized that physical grooming was replaced with communication in the hominin lineage as a mechanism to maintain strong bonds with a large number of social partners (Dunbar, 1996; Leavens et al., 2014). It is interesting that this relation exists in female bonobos, the more social and dominant sex. Further investigations into the relation between grooming and communication should be conducted in the other nonhuman great ape species – chimpanzees, gorillas, and orangutans.

Regarding joint attention findings, individuals that were more communicative with conspecifics during behavioral observations completed the receptive joint attention task

significantly faster than individuals that were less communicative (Figure 4.2.1). In addition, the number of external gestures produced during behavioral observations were positively associated with the number of communicative signals produced during initiating joint attention trials. Collectively, these are the first evidence of a direct relation between observable socio-communicative behavior and functional social cognition in bonobos. In addition, these results are consistent with findings in young children. Specifically, researchers have demonstrated that receptive joint attention skills are linked to later social and linguistic development (Markus et al., 2000). Together, these findings help identify promising behavioral and cognitive factors underlying socio-communicative development in humans, and nonhuman great apes.

It is unfortunate that in our sample of bonobos, there was no individual variability in the nucleotides present at the sites of known SNPs in humans that have been implicated in ASD. Sadly, many of the buccal swabs were insufficient for Sanger Sequencing and were not included in the visualization analysis. Therefore, the most subjects included for a given human SNP visualization were 9 bonobos. It is possible that we may have seen polymorphisms at these sites if there had been more bonobos from largely unrelated populations in this sample. However, given the human prevalence for the less common allele for several of the SNPs ranges between 13-41%, we would expect to see at least one bonobo from our sample with a different nucleotide at these sites, if in fact these were also SNPs in bonobos (Tansey, et al., 2011; Zhang, Zhao, Guo, Jones, Liu, et al., 2018). It is important to note that for 4 out of the 10 target variants, bonobos exhibited the alternate (risk) human allele (*FOXP2* rs6980093, *OXTR* rs237877, *OXTR* rs237878, *OXTR* rs237895). In humans, the alternate allele for these SNPs have been linked to the socio-communicative deficits of ASD (Baribeau et al., 2017; Cataldo et al, 2018; Mozzi et al., 2017) and are prevalent in 22-41% of the human population (Table 3.4.1). Surprisingly, although

several of the bonobos in this sample were unrelated, there was no observed individual variability within these variants in bonobos. However, given the high prevalence of these risk alleles in the human population, their implication in ASD, as well as the fact that all of the bonobos in this sample contained the risk allele, indicate that further investigation into *FOXP2* rs6980093, *OXTR* rs237877, *OXTR* rs237878, *OXTR* rs237895 is necessary. In other words, the fact that our (admittedly small) sample of bonobos contained this alternate human allele, and the fact that bonobos do not have language, suggest that these particular human SNPs may be a fruitful area for future investigations. Indeed, researchers may be able to better tease apart the influence of biological, behavioral, and cognitive mechanisms on socio-communicative development by studying these processes in bonobos.

Although there is no evidence to suggest that the 10 target SNPs are also polymorphic in bonobos, visualizations with the UCSC Genome Browser suggest there is a previously unidentified SNP in *OXTR* for our sample of bonobos - rs201778590. Specifically, the most common human allele for rs201778590 is G, and the only known bonobo allele is G (Table 4.3.1). However, findings from this study indicate an adult female bonobo (Lady) from CGZ has the alternate allele (A). It is important to note that Lady was wild born and is considered a founder individual for the entire captive bonobo population. In addition, Lady is the mother to several of the bonobos in the United States, including one of the other bonobos in the sample for the genetic analyses. In humans, rs201778590 is a missense variant, meaning it changes the amino acid that is produced (Veras, Getz, Froemke, Egidio Nardi, Sousa Alves, et al., 2018). The prevalence of an A allele for rs201778590 in humans is extremely rare and very little is known about the behavioral correlates of this allele. Certainly, additional investigations into the function and frequency of the alternate allele (A) in bonobos and humans are necessary. However, this

finding is the first evidence of a potential SNP in the bonobo *OXTR* and could provide valuable insights into the genetic underpinnings of great ape socio-communicative behavior.

Clearly, genetic variants are not the only factors that can impact socio-communicative behavior and social cognition in bonobos, humans, and other animals. Therefore, it is critical to consider potential environmental and biological factors that may account for the observed individual variability in socio-communicative behavior. To this end, I conducted a MANOVA analysis and found that both facility and sex had a significant main effect on aspects of socio-communicative behavior. Specifically, social proximity score, conspecific communicative production, and the number of gestures produced toward the experimenter (external gestures) differed significantly between facility. For example, the bonobos living at Ape Initiative (AI) communicated more often with conspecifics than did the bonobos living at Columbus Zoo and Gardens (CZG). One potential explanation for this finding is that two female bonobos, Mali and Clara, emigrated to AI in mid/late 2019 (one year before the observation period). Until this point, the AI bonobos only had one female in their social group. This is very atypical for bonobos, as the species is matriarchal and female-female bonding is extremely important. The addition of two new social partners, and a change toward a more typical, multi-female, multi-male social grouping, may have influenced the overall socio-communicative behavior of the AI bonobos. In contrast, CZG and MCZ did not have any newly integrated bonobos (within two years) preceding the observations. This higher production of communicative signals in the AI bonobos, as compared to the CZG (and to a lesser extent MCZ) bonobos, suggests communicative strategies are sensitive to aspects of the social environment, and provides evidence for the social complexity hypothesis for communicative complexity (SCHCC) that proposes that individuals living in complex social groups exhibit more elaborate communicative strategies (Bouchet,

Blois-Heulin, & Lemasson, 2013; Freeberg, Dunbar, & Ord, 2012; Krams, Krama, Freeberg, Kullberg, & Lucas, 2012).

In addition to a main effect of facility, the analyses also revealed a significant interaction between sex and facility for the number of gestures produced toward the human experimenter (external gestures). Specifically, male bonobos living at AI produced the most gestures toward the experimenter during behavioral observations. This could be due to several factors. For example, I have acted as a caregiver to the AI bonobos for almost seven years and have extensive experience feeding them, shifting them, running research tasks with them, and building trusting relationships with them. In contrast, I have had only limited experience working directly with the MCZ bonobos, and no personal experience with the CZG bonobos beyond collecting observations. The present personal relationship with the AI bonobos, as compared to the bonobos living at MCZ or CZG, may in part explain their proclivity to try and engage with the experimenter (me) during the behavioral observations. Another potential explanation for the high rates of external gestures produced by the male bonobos at AI is that three out of the four of these males (Kanzi, Nyota, and Teco) were raised, at least partially, in a human-oriented and language-rich environment. Indeed, researchers have found that great apes raised in these environments performed better on social cognition tasks and exhibited superior communicative abilities to great apes raised in standard captive settings (e.g., a zoo, sanctuary, or laboratory; Carpenter, & Tomasello, 1995; Leavens, & Bard, 2011; Russell, Lyn, Schaeffer, & Hopkins, 2011). This may also explain why there was an observed association between the number of external signals produced during behavioral observations and the number of communicative signals produced towards the experimenter during the initiating joint attention trials.

Collectively, these findings suggest that bonobos raised by humans in language-enriched environments frequently solicit the attention of humans in both typical and experimental settings.

However, there are a number of potential limitations regarding the behavioral observations and cognitive assessments that should be considered further (see Appendix). For example, given the limited access to captive bonobo populations, I had to take advantage of observations from the CGZ bonobos that were recorded during 2016/2017 using an almost identical ethogram to this project without the inclusion of abnormal/repetitive behaviors. Therefore, audio/video observations from the CGZ bonobos had to be coded post hoc for abnormal/repetitive behaviors. All MCZ and AI observations were live coded for the full set of socio-communicative measures. Although this is only a small methodological difference, it is possible that not all abnormal/repetitive behaviors were recorded for the CGZ given there is less visibility in videos than during live coding.

A more substantial limitation of this project is that there was very little social and environmental variability across data collection periods for bonobos living at MCZ, whereas bonobos living at AI experienced much more social and environmental diversity. For example, one MCZ social group consisting of a single adult male, an adult female, and her two juvenile female offspring did not vary in social composition for any of their combined 32 focal follows and were housed in the same indoor holding enclosure for a majority of their observations. On the other hand, a female bonobo living at AI was observed in six different social group compositions and in five different indoor and outdoor enclosure combinations. This lack of social and environmental diversity at MCZ as compared to AI may, at least in part, explain the observed differences in communicative production between the two facilities. Furthermore, two bonobos from MCZ, a 3-year-old male and a 7-year-old female were removed from their social

groups due to a medical emergency and remained out of their socials group for all the May 2021 focal observations (~half of the observations at MCZ). The removal of an infant male and a juvenile female could have had serious consequences on the socio-communicative behavior of the other bonobos living at MCZ. Together, the lack of social and environmental diversity at MCZ, as well as the removal of key social partners from the group, may explain why MCZ bonobos communicated less often than the AI bonobos, and spent less time in close proximity to conspecifics as compared to CGZ bonobos.

An additional limitation of the current study includes the small sample size for comparing observable socio-communicative behavior with performance on joint attention tasks (n=7). Specifically, it was only possible to collect joint attention assessments from the seven bonobos living at AI – separating individuals for joint attention testing was not possible at CGZ or MCZ. Ideally, the project would have included receptive and initiating joint attention assessments from all 26 bonobos living at AI, CGZ, and MCZ. If these critical subjects would have been included in the analyses, it is possible that additional associations between aspects of socio-communicative behavior and joint attention abilities, as well as between abnormal/repetitive and joint attention abilities, would have emerged.

For future studies, researchers should include more elaborate measures of social behavior and communication, such as social network analyses, to see how individual-level social communication interacts with various group dynamics. It would be very interesting to see if increased social complexity could necessitate more elaborate communicative strategies within a single species. In addition, future investigations should incorporate more fundamental measures of social cognition, like social orienting, to better understand how socio-communicative behavior is related to functional social cognition. Furthermore, researchers interested in early detection

and interventions for neurodevelopmental disorders, such as ASD, should consider investigating the efficacy of socio-cognitive training in relevant nonhuman models. Indeed, focusing on individual differences, and training competencies that form the foundation of higher-order social cognition in bonobos – the most social and communicative nonhuman species of great ape – could offer valuable insight into human socio-communicative development and provide promising evidence for attention-based intervention techniques aimed at improving socio-communicative abilities.

All told, there is an evident relation between aspects of social behavior, communication, abnormal/repetitive behaviors, and social cognition in the species most closely related to humans – the bonobo. Indeed, the aforementioned findings suggest that bonobos are the ideal species for testing hypotheses about typical and atypical socio-communicative development in humans. In addition, the results of this study are the first evidence of a potential SNP in *OXTR* of bonobos. Furthermore, this study fills a critical gap in our understanding of the various behavioral, cognitive, and genetic factors underlying socio-communicative development in humans, and our closest living relatives.

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APPENDIX

Appendix A. General Project Limitations

Appendix A.1 – Subject Availability and Facility Limitations

There are eight facilities in the United States that house bonobos. These include the Ape Initiative, Cincinnati Zoo and Botanical Garden, Columbus Zoo and Gardens, Fort Worth Zoo, Jacksonville Zoo and Gardens, Memphis Zoo, Milwaukee County Zoo and the San Diego Zoo. Unfortunately, each facility varies greatly in the number of bonobos, male:female sex ratio, age distribution, as well as enclosure size and access. For example, Memphis Zoo houses 7 bonobos, none of which are an adult male. This is very atypical for bonobo social groups in the wild and in captivity. Indeed, there are several captive male bonobos that are considered surplus. In contrast, Milwaukee County Zoo is home to 19 bonobos (8 males, 11 females) that range from 4 to 54 years old. Ideally, all of the bonobos in the U.S. would have been included in this study (N=89). Unfortunately, there were a number of reasons that data were only collected from three of these facilities – Ape Initiative (AI), Milwaukee County Zoo (MCZ), and Columbus Zoo and Aquarium (CGZ).

Out of these eight facilities, data collection for the entire project was only feasible at one of them – Ape Initiative (AI). For example, one facility cited understaffing and the amount of time necessary for the project as the factors that ultimately led to the review committee denying the project. In addition, many of the facilities do not allow access to "holding" or home enclosures for non-zoo personnel (required for joint attention testing). Indeed, shifting and separating individuals for joint attention assessments limited the facilities where the project could be carried out. The bonobos at AI are shifted and separated voluntarily for research and training on a daily basis. I am certified to shift and separate the AI bonobos which made data

collection much easier and less time consuming at this facility, as compared to others. Alternatively, shifting and separating bonobos for joint attention assessments was not possible at the other facilities. Furthermore, whole blood samples were very difficult to obtain. Only six banked blood samples were available (CGZ) for the 26 subjects. For the rest of the bonobos, buccal swabs had to be collected.

Appendix A.2 – Biological Sampling Limitations

Given banked blood samples were not available for most of the subjects, biological samples had to be collected in the form of buccal swabs. Although buccal swab collection is relatively easy to train in bonobos, and the materials are inexpensive, collecting biological samples this way poses several problems. Whole blood samples, as compared to buccal swabs, were much easier to process, produced clearer gels, and were more successful for Sangur Sequencing. On the other hand, many of the buccal swabs were insufficient for Sangur Sequencing (e.g., poor priming or high background). For the AI samples, buccal swabs were collected first thing in the morning before the bonobos had any food items. For the MCZ samples, swabs were collected opportunistically through the day. This may at least in part explain why more of the AI buccal samples worked for Sangur Sequencing, as compared to the MCZ samples. It is possible that given bonobos use their mouths to eat, groom one another, and drink liquids, and to a lesser frequency engage in urine drinking and coprophagia, the buccal swabs may have been contaminated with foreign DNA – making it very difficult to process the samples for Sangur Sequencing.

Appendix A.3 – Collecting Dissertation Data During a Global Pandemic

Due to the COVID-19 pandemic, data collection at zoos had to be put on hold for 14 months. Bonobos are highly suspectable to respiratory illnesses, including COVID. Therefore, access was restricted to zoo personnel. In addition, all university travel was suspended. This significantly impacted the timeline and scope of my dissertation project.

Specifically, to start data collection at Ape Initiative, I was required to quarantine for two weeks and provide proof of negative TB test. Due to their strict COVD-19 procedures, I had to quarantine on multiple occasions and provide proof of a negative TB test on multiple occasions throughout the data collection period. In addition, to resume data collection at Milwaukee I had to provide proof of a COVID-19 vaccine, given not all bonobos would be accessible from the public viewing space. Furthermore, obtaining approval to collect even observational data in zoos was extremely difficult during the pandemic. Indeed, many of the facilities drastically cut staff and were unable to accommodate any research at this time. All told, losing out on over a year of data collection time greatly impacted my dissertation project.